BIOCHEMICAL NOTES:

LABORATORY WORK

[FIRST AND SECOND PARTS]

BY

WILLIAM J. GIES

NEW YORK
1906

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PREFACE.

This volume gives a bare outline of the *laboratory work* of the course in *physiological chemistry* prescribed for first year students of medicine, at Columbia University, during the second half-year.

The work of preparing the volume for publication was begun in January, 1906. The "first part" was completed before the beginning of the course a few weeks later. It is my intention to prepare and issue additional parts from time to time, as more urgent university duties will permit.

The book is not intended to reduce in any degree the amount of personal instruction heretofore given by us, to each member of the class, without the help of such printed directions. We hope it may enable us to increase such personal attention. The student is obliged to think for himself as much as possible, under guidance, and is required to prepare a complete set of notes on the phenomena brought to his attention. The nature of the directions in this volume and our discussions of the experiments before and after completion, are expected to make it impossible for the student to go through his work mechanically. The laboratory work is supplemented by lectures, recitations and many demonstrations.

In this volume the capital letter **P** is used to signify the author's "Chemical notes: *physical and inorganic*" (1904). The numerals following this letter are intended to refer to *sections* of the volume indicated. The capital letter **O** is used in the same way to designate the volume of "Chemical notes: *organic*" (1904–'05). The capital letter **L** refers to the volume of "Chemical notes: *laboratory work*" (1905).

WILLIAM J. GIES.

LABORATORY OF PHYSIOLOGICAL CHEMISTRY,
COLLEGE OF PHYSICIANS AND SURGEONS,
February 1, 1906.

CONTENTS.

[First Part.]

Estimated Percentage Elementary Composition of the Earth's Crust Including Air and Water [Clarke].

1. Oxygen	49.98		75.28	99.07
2. Silicon	25.30			
3. *Aluminium*	7.26	...12.34	22.85	
4. Iron	5.08			
5. Calcium	3.51	...10.51		
6. Magnesium	2.50			
7. Sodium	2.28			
8. Potassium	2.22			
9. Hydrogen	0.94			
10. *Titanium*	0.30		0.92	
11. Carbon	0.21			
12. Chlorin	0.15			
13. Phosphorus	0.09			
14. *Manganese*	0.07			
15. Sulfur	0.04			
16. *Barium*	0.03			
17. Nitrogen	0.02			
18. *Chromium*	0.01			
19. *Fluorin, iodin, lead, copper, zinc, tin, mercury, arsenic, silver, gold, platinum* and *all others* (combined)	0.01			
	100.00			

Note. The elements indicated by italics are either entirely missing from nearly all organisms, or occur at most in only *very minute* proportions. See the adjoining table.

Estimated Elementary Composition of a Man. Height, 5 Ft. 8 In.; Weight, 148 Lbs. [Moss].

	Lbs.	Per Cent.			
1. *Oxygen*	92.40	62.29	83.60		99.40
2. *Carbon*	31.60	21.31			
3. *Hydrogen*	14.60	9.84	12.95	15.80	
4. *Nitrogen*	4.60	3.11			
5. Calcium	2.80	1.90	2.85		
6. *Phosphorus*	1.40	0.95			
7. Potassium	0.34	0.23			0.60
8. *Sulfur*	0.24	0.16			
9. Chlorin	0.12	0.08			
10. Sodium	0.12	0.08			
11. Magnesium	0.04	0.02			
12. Iron	0.02	0.01			
13. Silicon	0.02	0.01			
14. *Fluorin*	0.02	0.01			
15. Iodin	trace	.....			
16. Manganese	"	.....			
	148.32				100.00

Notes. *a.* Trifling quantities of aluminium, arsenic, bromin, copper, lead, zinc and a few other elements occur naturally in some organisms.

b. The elements indicated by italics occur in organisms in larger *relative* quantities than they do in the inorganic world. See the adjoining table.

General Classification of the Substances Contained in Plants and Animals, with the Names of Representatives of Each Group.

Inorganic.

Water.

Gases—free and in solution: carbon dioxid, CO_2.

Salts—precipitated and in solution:

a. Neutral: sodium chlorid, NaCl.

b. Acid: mono-potassium di-hydrogen phosphate, KH_2PO_4.

c. Alkaline: sodium carbonate, Na_2CO_3.

d. Cations and anions of all the inorganic substances: $NH_4^{\bullet}$, SO_4''.

Free acids—in solution: hydrochloric acid, HCl; gaseous: hydrogen sulfid, H_2S.

Cations and anions associated with *organic* radicals—$Ca^{\bullet\bullet}$, C_2O_4''.

Nearly all the *inorganic* substances referred to are present in small proportions in the food of *animals* and many are formed in whole or part in *animals* from *organic* substances by *analytic* processes.

Most of the inorganic substances referred to above are utilized in *plants* in processes resulting in the *synthesis* of *organic* substances.

Organic.

Primary. *Synthetic* products in organisms, made in *plants* especially:

a. Fats: tripalmitin, $C_{51}H_{98}O_6$; *fat-like substances;* lecithin, $C_{44}H_{90}NPO_9$.

b. Carbohydrates: starch, $(C_6H_{10}O_5)_x$

c. Proteins: albumin, $C_{450}H_{720}N_{116}S_6O_{140}$.

Secondary. *Analytic* products in the main, which are wholly or partly derived from the primary by metabolic processes, in *animals* especially:

a. Aliphatic: leucin, $C_5H_{10}(NH_2)COOH$.

b. Carbocyclic: phenol, C_6H_5OH.

c. Heterocyclic: indol, C_8H_7N.

d. Products of unknown chemical character: enzymes.

This simple and very general classification should be kept clearly in mind throughout all our work in biological chemistry.

CHAPTER I.

INORGANIC MATTERS.

A. Demonstrations. Water and Inorganic Solids in Typical Animal and Vegetable Products, such as Blood, Muscle and Potato:

1. Distillation of contained liquids and detection of water in each distillate. 2. Quantitative determination of water. 3. Inorganic residue obtained by incineration. 4. Quantitative determination of ash. 5. Distinction between preformed inorganic matter and inorganic matter derived from organic matter by incineration.

B. Percentage Amounts of Water and Ash Obtained from some Mammalian Parts.

6. In the subjoined table are given figures representing the average proportions of water and ash obtainable from typical mammalian liquids and solids under normal conditions.

	Water.	Ash.		Water.	Ash.
Saliva	99.5	0.2	Spleen	72.0	0.6
Gastric juice	97.3	0.7	Brain:		
Bile	97.0	0.8	Gray matter	83.5	1.5
Lymph	95.5	0.7	White matter	70.0	0.6
Semen	90.3	0.9	Tendon	62.9	0.5
Kidney	81.1	1.0	Ligament	57.6	0.5
Blood	79.5	0.8	Bone	30.0	43.0
Muscle	76.5	1.0	Dentin	10.0	66.0
Liver	76.2	1.0	Adipose tissue	4.4	0.2
Cartilage	73.6	1.5	Enamel	Trace	96.0

C. Leading Inorganic Ions in Biological Liquids:

7. **Cations.**—$H^{\cdot}$, $NH_4^{\cdot}$, $Na^{\cdot}$, $K^{\cdot}$, $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$.

8. **Anions.**—OH', Cl', SO_4'', PO_4''', HPO_4'', H_2PO_4', CO_3'', HCO_3'.

D. METHODS FOR THE DETECTION OF THE MOST IMPORTANT INORGANIC CATIONS AND ANIONS IN TYPICAL BIOLOGICAL LIQUIDS * SUCH AS BLOOD, URINE AND FRUIT JUICE. † (L: 92–192.)‡

9. Hydrion, H˙, and hydroxidion, OH′. Determine the reactions (L: 58–65) of the biological liquids to litmus, lacmoid and phenolphthalein (67).

10. Metals (cations). With the exception of iron, heavy metals occur only very rarely, or in only very minute proportions in biological products, and special methods as well as relatively large quantities of material are required for their detection. Wherever in normal biological products iron and other heavy metals may be found, the metallic atoms exist, as a rule, in complex molecular combinations and occur very rarely in ionic or dissociable forms.§

*Biological solids may ordinarily be dissolved without particular difficulty. Aqueous or acid solutions or extracts usually contain ions fully representative of all the inorganic substances present in the original material. See the succeeding footnote.

† The plan of qualitative analysis presented in this chapter will enable the student to prove definitely the *absence* of some common cations and anions, as well as to demonstrate the *presence* of certain others. This intention has prevented the brevity that might otherwise have been sought in the scheme of processes here outlined. See 32–35.

‡ See preface.

§ Various *organic* substances of common occurrence in biological products, such as oxalic acid, citric acid, tartaric acid, sugars, purin bases, and proteins, interfere more or less with the detection of certain cations, especially of some of the heavy metals. Thus, hydrochloric acid precipitates a number of proteins, *e. g.*, mucoids, in masses that resemble superficially the precipitates of the metals of the first group. Oxalic acid interferes with the precipitation of tin as sulfid by hydrogen sulfid and favors the precipitation of earthy metals as oxalates, with metals of the third group as hydroxids, when their mixed solutions are rendered alkaline with ammonium hydroxid (15). Such instances might be multiplied.

When it is desired to detect *traces* of *heavy metals* as cations, or larger proportions "combined," in biological materials, it is necessary as a preliminary step in the application of the customary methods of inorganic analysis to *destroy the organic matter.* This may be accomplished in several ways. Thus, the product may be acidified strongly with nitric acid, the acidified material evaporated to dryness and the dry residue ignited *very gently* until no further odor of burning material can be detected. The nitric acid favors the conversion of any iron to the ferric form and aids in the destruction of the organic matter. Complete *charring* is sufficient at this stage and *high heat must be avoided* in order to prevent conversion of iron, particularly, into a very insoluble oxid. The black residue should be thoroughly extracted with warm nitric acid and the extract filtered. The washings should be added to the main filtrate. The carbonaceous matter on

11. First group. To about 5 c.c. of the original (filtered?) liquid add drop by drop a moderate excess of dilute hydrochloric acid. Preserve the liquid for use in process **12**. Hydrochloric acid and other acids precipitate a number of common *organic* biological compounds,* to which attention will be drawn later. See footnote, page 8. Silicates are rarely if ever present in biological liquids

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the filter may be completely incinerated at a relatively low temperature, with the aid of nitric acid, and a second warm acid extract obtained for the recovery of residual traces, which should be added to the previous extract. A silicious residue may remain. The combined acid extracts should be evaporated to *complete* dryness on a *water-bath*, until no further odor of nitric acid can be detected on stirring. *Ignition must be avoided.* The residue should be treated with a little concentrated nitric acid, diluted with water and boiled. The residue dissolves completely, or a white deposit of silicic oxid remains. To the combined filtrate and washings ammonium hydroxid should be added until the solution is rendered neutral or very nearly neutral. To this solution may be applied directly the successive operations outlined above (11–35), except those for the detection and removal of oxalic acid. More detailed methods than those given above (11–35) must be used for the identification of metals of unusual biological occurrence that might be present. Such methods are given in the author's volume on laboratory work in general chemistry (**L**: 92–158), and in most volumes on qualitative inorganic analysis.

The method of eliminating organic matter that has just been described usually results in the *production* of *inorganic* matter from *organic* non-electrolytes, and on that account does not give a true indication of the character of preëxistent ions or dissociable compounds when they occur. (Demonstration 5.)

* If a precipitate was produced by hydrochloric acid, filter and subject the precipitate to the following treatment in order to show the presence or absence of metals of the first group — silver, mercury (ous), lead. Wash slightly with *cold* water. Reject the washings. Treat the precipitate on the filter with about 25 c.c. of *hot* water. Pour the *filtrate* on the precipitate several times (boil each time before doing so) to insure complete solution of any lead chlorid that may be present. Filter. Cool. Test for lead in the filtrate by adding potassium iodid or dilute sulfuric acid. (**L**: 105.)

Wash the residue with *hot* water. Reject the washings. Treat the residue on the filter with about 25 c.c. of *warm* ammonium hydroxid. Pour the *filtrate* upon the precipitate several times (*warm* each time before doing so) to insure complete removal of any silver chlorid that may be present. Mercurous chlorid is converted by this treatment into a *black* solid mixture of metallic mercury and mercuric ammonium chlorid. A white residue at this point probably consists of albuminous matter. Filter. Any silver chlorid originally present in the precipitate would be contained in the ammoniacal filtrate in the form of ammonio-silver chlorid. Albuminous matter would also dissolve somewhat. Acidify the filtrate with nitric acid. If a white precipitate is formed in the *acid* liquid, silver as silver chlorid is indicated. Silver chlorid gradually darkens in diffused sunlight and does not blacken on ignition. A white precipitate of albuminous matter that might occur at this point is not affected by sunlight but blackens and disappears on ignition. (**L**: 105.)

in sufficient proportions for silicic acid to be precipitated by hydrochloric acid. See 39.

If a precipitate was produced on adding hydrochloric acid, observe whether sodium chlorid or any other soluble chlorid brings about the same result in a new portion of the liquid.*

12. Second group. As a rule nearly all the metals of the second group are entirely absent from normal biological liquids. Although traces of arsenic, for example, appear to be normally present in practically all animals and copper is contained in many, the proportions in which these elements occur are too trifling for detection by ordinary means (footnote, page 8). Besides, wherever arsenic and copper exist in animals ordinarily, they occur as a rule in organic, *non-dissociable* combinations.

13. Warm the clear acid liquid (**11**), or the filtrate from (or extract of) any precipitate that may have been formed by hydrochloric acid. Add a drop of hydrochloric acid to make certain that previous (?) precipitation was complete, filter if necessary and treat the solution with a slight excess of hydrogen sulfid, preferably in aqueous solution. Cool. Proceed to **14.**

14. Third group. *Preliminary examination for phosphate and oxalate.* Phosphate occurs almost universally, oxalate occasionally, in biological materials. Oxalate is always accompanied in organisms by phosphate. The presence or absence of phosphates and oxalates determines, as a rule, the method to be employed for the detection of the metals of the third, fourth and fifth groups.†

15. *Precipitation of phosphates and oxalates.* To about 25 c.c. of the *original* solution ‡ add a few drops of *dilute* nitric acid and boil for about a minute. Filter off, wash and *reject* any precipitate

* Any albuminous matter that might be precipitated by hydrochloric acid would remain in solution on addition of sodium chlorid instead of hydrochloric acid. On the contrary the metals of the first group would be precipitated. See footnote, page 9.

† Silicates and fluorids, which occasionally occur in minute proportions in biological liquids, affect slightly the method of procedure at this point. Their influence need not be taken into account, however, except in very special cases.

‡ Blood and other albuminous liquids should be diluted with several volumes of water before adding the nitric acid. If any of the metals of the first or second groups were detected in tests 11 and 13 they must be removed from the solution before proceeding to the separation of the metals of the third, fourth and fifth groups. After the elimination of such heavy metals hydrogen sulfid should be *completely* removed from the final filtrate by boiling. Determine this matter accurately by testing the vapor with wet "lead acetate paper." The solution is then in proper condition for the treatment indicated above, under 15.

(albuminous) that may be produced at this point. Treat the acid solution (filtrate?) with about an equal volume of ammonium chlorid solution and a slight excess of ammonium hydroxid. Boil until the odor of ammonia can hardly be detected. Filter the slightly alkaline, precipitated mixture.* Wash. *Retain the filtrate and washings for examination later* (24) and proceed with the precipitate as follows (16–19).

16. *Detection of phosphate.* Dissolve a small portion of the precipitate in a little nitric acid. Add about an *equal volume* of "molybdic solution" (ammonium molybdate in nitric acid†). Warm gently to about the temperature of the body and let the mixture stand under observation until after the conclusion of the next test. Typical equation:

$$12NH_4HMoO_4 + Na_2HPO_4 + 11HNO_3 = (NH_4)_3PO_4(MoO_3)_{12} + 9NH_4NO_3 + 2NaNO_3 + 12H_2O$$

Ammonium phospho-molybdate (yellow)

If an appreciable quantity of phosphate is present, a yellow precipitate will form in a few minutes.‡

17. *Detection of oxalate.* Treat a portion of the precipitate with a moderate excess of sodium carbonate and boil for a minute or two. Filter. Acidify the filtrate slightly with *acetic* acid and add calcium chlorid. Typical equation:

$$CaCl_2 + Na_2C_2O_4 = CaC_2O_4 + 2NaCl$$

Calcium oxalate (white)

The white pulverulent precipitate of calcium oxalate forms quickly in the presence of even traces of oxalate.

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*The precipitate obtained at this point in biological liquids usually consists chiefly of earthy phosphates, almost invariably of calcium phosphate and ammonio-magnesium phosphate; but inorganic iron, as well as manganese and other metals of the third and fourth groups that might have been contained in the original solution, would also be present as hydroxid or phosphate or both; and, if oxalate occurred in the original solution, earthy oxalates, chiefly calcium oxalate, might also be contained in the precipitate. See the footnote on page 8. Silicates are present in biological liquids in proportions that are too slight to yield precipitates of silicic acid on adding ammonium hydroxid (39).

†Ammonium molybdate solution or so-called "molybdic solution" is usually made in the following proportions: 100 grams of molybdic anhydrid are dissolved in 417 c.c. of ammonium hydroxid (sp. gr. 0.96). This solution is poured in small quantities at a time into 1250 c.c. of nitric acid (sp. gr. 1.20). The acid solution is thoroughly shaken after each addition of the alkaline liquid. The mixture is kept in a warm place for several days. The filtrate is the reagent.

‡Arsenate could not be present in the solution. See 13, also 45.

18. *Detection of metals of the third group (iron).* Of these iron is the only one of particular biological importance. It can seldom be detected directly in biological liquids.

19. If oxalate was *absent* (17) proceed to 20. If *both* oxalate and phosphate were detected (16–17) in the precipitate (15), the remaining portion of the precipitate (with the filter paper if necessary) should be transferred to a porcelain crucible or a platinum foil, dried and very *gently* ignited. The oxalic acid will be destroyed by combustion. Proceed to 20.

20. This ignited residue (19), or the main bulk of the original precipitate (15) if oxalate was absent, should be dissolved in a small amount of hydrochloric acid. Proceed to 21.

21. *Iron.* Dilute with water a small quantity of the acid solution just prepared (20) and add to it potassium ferrocyanid or ammonium sulfocyanate. As a rule the test is negative, unless an ash is the material undergoing analysis. Use the main bulk of the solution in process 22. Equations:

a. $$4FeCl_3 + 3K_4Fe(CN)_6 = \underset{\substack{\text{Ferric ferrocyanid}\\(\text{“Prussian blue”})}}{Fe_4[Fe(CN)_6]_3} + 12KCl$$

b. $$FeCl_3 + 3NH_4SCN = \underset{\substack{\text{Ferric sulfo-}\\\text{cyanate}\\(\text{red; soluble})}}{Fe(SCN)_3} + 3NH_4Cl$$

22. *Removal of metals of the third group (iron), and also of phosphate.* Add sodium hydroxid to the remainder of the acid solution (20) until the latter is nearly neutral. Treat the resultant slightly acid liquid with a solution of sodium acetate strongly acidified with acetic acid and heat the mixture gently for about 10 minutes, to remove excess of acetic acid. If iron was present ferric phosphate will be precipitated. To the solution add ferric chlorid drop by drop until the liquid assumes a red color. This coloration indicates that the iron which was introduced has united with all the *phosphate* in the solution and has begun to combine with the associated acetate. Heat gently for about 10 minutes. Filter while the mixture is warm. Wash with hot water. Reject the precipitate, which consists of phosphate and acetate of iron.* The filtrate and washings may contain cations of any metals of groups

*Much, if not all, the iron obtained at this point, it will be recalled, was purposely added to the solution for the *removal of phosphate.*

four and five that were contained in the original precipitate (15). It may also contain a trace of iron that was not precipitated in the foregoing process. A method for removing such a trace of iron is indicated below (24). Proceed to 24.

23. **Fourth group.** Of this group manganese is the only one of general biological significance. It can seldom be detected by ordinary means because of the slight proportions in which it occurs. Like iron it occurs mainly if not wholly in organic combination. (See footnote, page 8.)

24. Mix filtrates 15 and 22. Reduce the volume one half by evaporation. Add to the concentrated liquid a moderate excess of ammonium hydroxid, and boil until nearly all ammonia has been eliminated. Filter, if any trace of iron, or anything else that had not been previously precipitated, should be thrown out of solution at this point. Add colorless ammonium sulfid, in slight excess. Heat to boiling. Filter if necessary.* Proceed to 26.

25. **Fifth group.** Of this group calcium is the only element of biological importance. It is a relatively prominent constituent of practically all organisms.

26. *Calcium.* The preceding filtrate (24) should be concentrated by evaporation and cooled. Excess of ammonium sulfid is expelled in this process. If a precipitate forms as a result of this treatment add just enough water to dissolve the precipitate.† Render the liquid alkaline with ammonium hydroxid and add

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* As a rule precipitation does not occur because of the absence of manganese and the remaining metals of the fourth group or because only the merest traces are present. If a precipitate is produced, however, test for manganese as follows: Dissolve the precipitate on the filter in dilute hydrochloric acid. To the filtrate add excess of sodium hydroxid. If manganese was present it will be precipitated as white manganous hydroxid which will gradually be converted into the brown manganic hydroxid. Put the filter and precipitate in a casserole and heat to boiling in nitric acid. Add several very small quantities of red lead (less than half a gram in all). Boil again for a moment. Filter. A pink filtrate indicates the presence of manganese. This color must be looked for immediately after filtering. When only a small proportion of manganese is present the faint pink color speedily disappears as a result of oxidation. The color may usually be seen on permitting the excess of Pb_3O_4 to subside. (L: 138.) Equation:

$$2Mn(OH)_2 + 5Pb_3O_4 + 30HNO_3 = \underset{\text{Permanganic acid (red)}}{2HMnO_4} + 15Pb(NO_3)_2 + 16H_2O$$

If manganese is present in the precipitate produced by ammonium sulfid, the precipitate will impart an amethyst-red color to a borax bead when it is heated with the latter on a platinum wire in an *oxidizing* flame. In a *reducing* flame the bead becomes colorless.

† If sulfur separates filter the mixture.

ammonium carbonate to it until precipitation is complete. Warm the mixture but do not heat to boiling. Filter off the precipitate of calcium carbonate and use the filtrate for the detection of magnesium in process 28. Confirm the presence of calcium in the washed precipitated carbonate by dissolving the precipitate in a slight quantity of acetic acid. In this acid liquid, ammonium oxalate causes precipitation of calcium oxalate (17). Let the mixture stand if precipitation does not occur immediately. Equation (17):

$$Ca(CH_3CO_2)_2 + (NH_4)_2C_2O_4 = \underset{\substack{\text{Calcium oxalate}\\\text{(white)}}}{CaC_2O_4} + 2NH_4CH_3CO_2$$

27. **Sixth group.** Magnesium, sodium, potassium (and ammonium) are present in practically all biological products.

28. *Magnesium.* Concentrate by evaporation the filtrate from the precipitated carbonate (26), and add moderate excesses of ammonium chlorid and ammonium hydroxid. Treat the solution with ammonium oxalate. Let the mixture stand several minutes. Filter off any calcium oxalate that may have been precipitated.* Add drop by drop to the filtrate a moderate excess of di-sodium hydrogen phosphate. Stir thoroughly. Let the mixture stand a few minutes. Equation:

$$MgCl_2 + Na_2HPO_4 + NH_4OH = \underset{\substack{\text{Ammonio-mag-}\\\text{nesium phosphate}\\\text{(white)}}}{NH_4MgPO_4} + 2NaCl + H_2O$$

In each of the remaining tests for cations (29–30) use portions of the *original* solution, as follows:

29. *Sodium and potassium. Flame tests.*† Use platinum wire. Persistent yellow indicates sodium. A violet color, not obscured by blue glass, indicates potassium.

30. *Ammonium.* Add sodium hydroxid in excess. Boil. Notice the character of any (*a*) *odor* that may be detected, also the reaction of the steam on (*b*) *wet litmus paper*, and the effect of the steam on (*c*) *hydrochloric acid* adherent to a glass rod. Equations:

a. $$NH_4Cl + NaOH = \underset{\substack{\text{Ammonia}\\\text{(gas)}}}{NH_3} + H_2O + NaCl$$

c. $$NH_3 + HCl = \underset{\substack{\text{Ammonium chlorid}\\\text{(white fumes)}}}{NH_4Cl}$$

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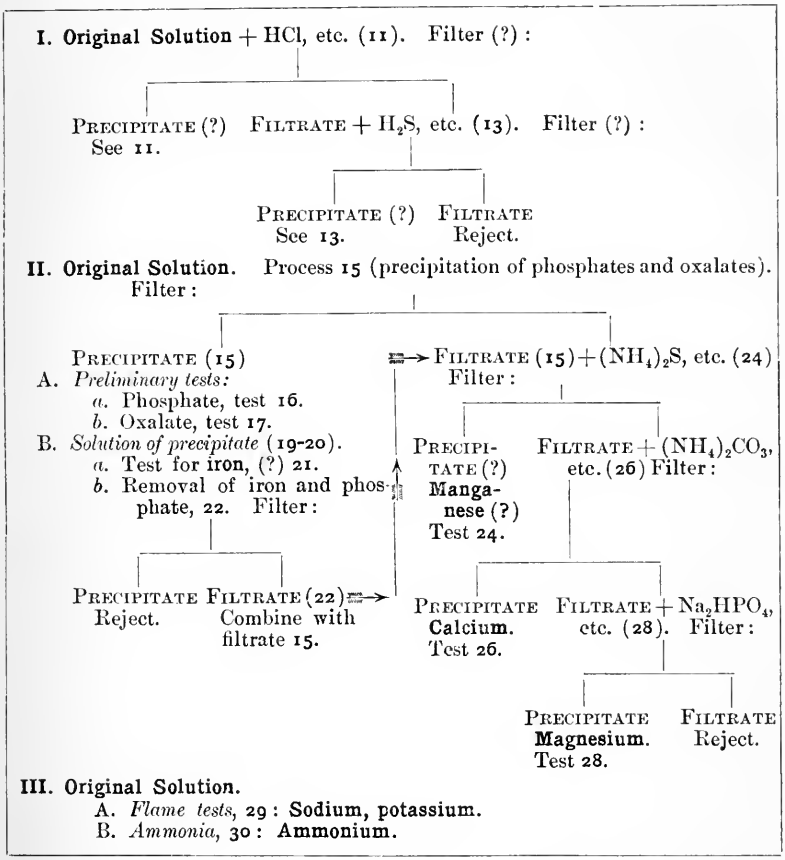
* Calcium is not completely precipitated by ammonium carbonate.

† Special methods are required in unusual cases. Sodium and potassium are invariably present in biological products.

31. Summary of cation processes, 9–30. In the subjoined summary the methods heretofore described for the detection of cations are indicated in a manner intended to favor easy review.



Methods for the detection of cations of metals of groups V and VI in the absence of cations of metals of groups I–IV. 32. Since calcium, magnesium, sodium, potassium and ammonium are the leading inorganic cations contained in biological liquids, and since the *cations* of the metals of groups **I–IV** are usually *absent*, the following plan of analysis leads to a more speedy detection of the cations of greatest biological importance.

33. Test for *sodium*, *potassium* and *ammonium* as was previously indicated (29–30).

Test for calcium and magnesium as follows: 34. *Calcium.* Make the solution alkaline with ammonium hydroxid. Earthy phosphate may be precipitated. To this mixture add *acetic* acid drop by drop until the reaction of the liquid is acid and all the precipitated phosphate has gone into solution. Filter if necessary. Treat the *acid* mixture with ammonium oxalate. Calcium oxalate will be precipitated (17). Heat the mixture to boiling in order to favor filtration. Filter. (Test the filtrate with ammonium oxalate to make certain that calcium was completely precipitated in the preceding treatment.)

35. *Magnesium.* To the filtrate (34) add excess of ammonium hydroxid and di-sodium hydrogen phosphate.* A white precipitate of ammonio-magnesium phosphate is usually formed (28).

36. **Acids (anions).** The salts that are of particular biological significance and which are represented by the corresponding inorganic anions are chlorids, phosphates, carbonates and sulfates. Silicates, fluorids and sulfids are less conspicuous. Trifling quantities of sulfocyanates, nitrates, nitrites and other inorganic salts also occur normally in various biological products.

37. Boil about 25 c.c. of the original solution † with a moderate excess of sodium carbonate.‡ If a precipitate is formed at this point it may contain carbonates, hydroxids, phosphates, silicates and fluorids. Filter, if necessary, while the mixture is hot, and in that case use the filtrate as indicated below (41–47) and the precipitate as follows:

38. Transfer all the precipitate (37) § to an evaporation dish, add sufficient nitric acid to acidify the resultant solution and evaporate it to dryness on a water bath. Treat the residue (39) on a hot water bath with dilute nitric acid and water, filter and wash (40).

39. *Silicate.* A white *gritty* residue at this point (38) consists of hydrated silicic acid. See 43. A *sandy* residue rarely remains at

* There may be sufficient PO_4 in the filtrate to make this addition of phosphate unnecessary and to yield a precipitate of ammonio-magnesium phosphate on addition of the ammonium hydroxid.

† See footnote, page 11.

‡ Heavy metals tend to prevent easy detection of some anions. Any heavy metals present in the liquids under examination are precipitated by the carbonate. As a rule this process is unnecessary because of the absence of cations of heavy metals. (11–13.)

§ A trace of fluorid may be present in the precipitate. When its detection is sought, a portion of the precipitate must be reserved for special tests for fluorin.

this stage of the process. Any albuminous matter that might be present would disappear on ignition; the silicic acid would remain (43).

40. *Phosphate.* Test the filtrate (38) for phosphate as in 16, but filter before adding molybdic solution, if the nitric acid produced a precipitate.

41. Divide into three unequal parts the unprecipitated alkaline solution obtained after the above-described treatment with sodium carbonate (37), or the alkaline filtrate from any precipitate that may have been formed. Tests should be applied to the parts as follows:

First part ($\frac{3}{5}$). Add a few drops of nitric acid. Boil to expel carbon dioxid. Add more nitric acid, if necessary, to effect permanent slight acidification. Albuminous matter may be precipitated. Filter if necessary and reject the precipitate. Then add ammonium hydroxid to make the reaction *slightly* alkaline. Boil until the odor of ammonia can no longer be detected in the vapor. Filter if necessary.* Reject the precipitate. Treat the filtrate as follows:

42. *Sulfate.* Acidify with hydrochloric acid. Add solution of barium chlorid. Equation:

$$Na_2SO_4 + BaCl_2 = \underset{\substack{\text{Barium sulfate}\\(\text{white})}}{BaSO_4} + 2NaCl$$

43. *Silicate.* If silicic acid has not been previously detected, acidify with hydrochloric acid, evaporate on a water bath to dryness and apply tests 38–39 to the residue. The test is almost always negative (39). Equation:†

$$Na_2SiO_3 + 2HCl = \underset{\substack{\text{Silicic acid}\\(\text{white})}}{H_2SiO_3} + 2NaCl$$

44. *Chlorid.* Acidify with nitric acid. Add solution of silver nitrate. Equation:

$$NaCl + AgNO_3 = \underset{\substack{\text{Silver chlorid}\\(\text{white})}}{AgCl} + NaNO_3$$

* A slight precipitate containing compounds of metals previously soluble in hot sodium carbonate solution (37), may be formed at this point. Albuminous matter may also be present.

† Dried to a dust on a water-bath (temperature below 100° C.) polysilicic acids, such as the one indicated in the following equation, are produced: $4H_2SiO_3 = H_2Si_4O_9 + 3H_2O$. Thoroughly dried at temperatures above 110° C., silicic anhydrid results: $H_2Si_4O_9 = 4SiO_2 + H_2O$.

45. *Phosphate.* Acidify with nitric acid and apply test 16 to the remaining portion of the filtrate (40).*

46. *Nitrate.* Second part ($\frac{1}{5}$) [41]. Evaporate to dryness in a casserole on a water bath. Moisten the residue with a moderate excess of phenol di-sulfonic acid,† and return the mixture to the hot water bath. While the mixture is being heated, stir it in order to favor complete disintegration of any solid organic matter. Cool. *Cautiously* add ammonium hydroxid *in small quantities* until the mixture becomes strongly alkaline. Filter. Typical equation:

$$\underset{\substack{\text{Phenol}\\ \text{di-sulfonic acid}}}{C_6H_3(OH)(SO_3H)_2} + 3NaNO_3 = \underset{\substack{\text{Picric acid}\\ \text{(yellow)}}}{C_6H_2(NO_2)_3(OH)} + Na_2SO_4 + NaHSO_4 + H_2O$$

$$C_6H_2(NO_2)_3(OH) + NH_4OH = \underset{\substack{\text{Ammonium}\\ \text{picrate}\\ \text{(yellow)}}}{C_6H_2(NO_2)_3ONH_4} + H_2O$$

A yellow filtrate indicates the previous presence of nitrate. The color of the filtrate is due to dissolved ammonium picrate. Biological liquids rarely contain nitrate and the result of the test is usually negative.

47. *Sulfid.* Third part ($\frac{1}{5}$) [41]. Acidify with hydrochloric acid. Test with moist lead acetate paper any gas that may be formed. Heat the mixture and continue the test. The result is usually negative. Equations:

$$Na_2S + 2HCl = \underset{\substack{\text{Hydrogen}\\ \text{sulfid (gas)}}}{H_2S} + 2NaCl$$

$$H_2S + Pb(C_2H_3O_2)_2 = \underset{\substack{\text{Lead sulfid}\\ \text{(black)}}}{PbS} + 2C_2H_4O_2$$

48. *Carbonate.* To a new portion of the *original* solution add hydrochloric acid to acid reaction. If effervescence occurs (*a*) re-

* To make certain that arsenic is not responsible for the yellow precipitate that may occur at this point (16) (if a precipitate was obtained with hydrogen sulfid in test 13), it is necessary to acidify the filtrate with hydrochloric acid and to treat the hot solution (70° C.) with hydrogen sulfid gas for about 30 minutes to precipitate arsenic. Filter, expel hydrogen sulfid from the filtrate by boiling, add nitric acid and finally molybdic solution. As a rule this procedure is quite unnecessary because of the absence of arsenic in quantities sufficient for detection (13, 16).

† The reagent was prepared in the following proportions: 30 grams of phenol and 370 grams of sulfuric acid (1.84 sp. gr.) were mixed and heated at about 100° C. for six hours on a water bath.

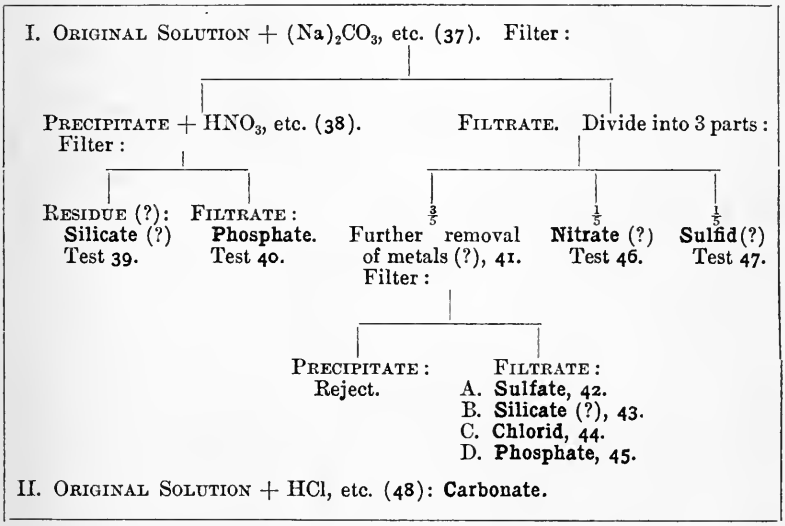
peat the test with a sufficient volume of the liquid in a closed flask connected with lime-water under a layer of kerosene. Heat the acidified mixture. If a white precipitate forms in the lime-water (*b*), test its solubility and effervescent properties in dilute acetic acid (*c*). Equations:

a. $Na_2CO_3 + 2HCl = \underset{\text{Carbon di-oxid (gas)}}{CO_2} + NaCl + H_2O$

b. $Ca(OH)_2 + CO_2 = \underset{\substack{\text{Calcium}\\ \text{carbonate}\\ \text{(white)}}}{CaCO_3} + H_2O$

c. $CaCO_3 + 2C_2H_4O_2 = CO_2 + Ca(C_2H_3O_2)_2 + H_2O$

49. Summary of anion processes, 36–48. In the summary on the opposite page the methods heretofore described for the detection of anions are indicated in a manner intended to favor easy review.



E. METHODS FOR THE DETECTION OF THE LEADING ELEMENTS THAT OCCUR IN BIOLOGICAL ORGANIC COMPOUNDS.* (L: 201–207.)

50. Carbon. Ignite in an evaporation dish about 10 grams of pulverized cupric oxid, CuO. Cool to the temperature of the body

*Oxygen is not referred to in this section because there are no satisfactory methods for its qualitative determination in organic compounds. It may be determined by certain quantitative processes.

and then mix with it about a gram of dry pulverized cane sugar, $C_{12}H_{22}O_{11}$. Transfer to a *dry* hard glass test tube provided with a perforated cork with L-tube and gradually ignite. Conduct the gas into lime-water under a layer of kerosene (48). Equation :

$$C_{12}H_{22}O_{11} + 24CuO = \underset{\text{Carbon dioxid (gas)}}{12CO_2} + 11H_2O + 24Cu$$

Notice the metallic copper that resulted. Read **51** before emptying the tube.

In this process *organic* carbon is oxidized to carbon dioxid.

51. Hydrogen. After the mixture in the tube (50) has been thoroughly ignited, allow it to cool. Examine it carefully. Remove with a stirring rod some of the liquid that condensed in the upper part of the tube and bring it in contact with anhydrous copper sulfate. Equation :

$$\underset{\text{Cupric sulfate anhydrous (white)}}{CuSO_4} + 5H_2O = \underset{\text{Cupric sulfate hydrous (blue)}}{CuSO_4, 5H_2O}$$

In this process (50) *organic* hydrogen is oxidized to water.

52. Nitrogen. *Detected as ammonia.* To a small quantity of dry pulverized egg albumen in a mortar add about ten to twenty times the amount of powdered soda lime. Mix the two thoroughly, transfer the mixture to a *dry* test tube and ignite. Apply test **30** to the gas evolved.

In this process nitrogen in *organic* combination is converted into ammonia.

53. *Detected as cyanid.* (Lassaigne's test.) Into a *dry* test-tube place a dry piece of metallic sodium the size of a pea and about twice as much dry pulverized blood. Ignite. Raise the temperature gradually and maintain a glow for a minute or two. Dip the hot end of the tube in 5 c.c. of water in an evaporation dish. The tube will break and its contents will pass into the water. Warm the mixture to favor solution of soluble products. Filter through a very small filter. Add several drops of solutions of sodium hydroxid and *ferrous* sulfate, and a single drop of *ferric* chlorid solution. Boil for a minute, then cool the mixture. Finally add just enough dilute hydrochloric acid to dissolve the precipitated ferrous and ferric hydroxids. The clear solution becomes

bluish green. If the result is negative at first let the mixture stand. A precipitate of "Prussian blue" will gradually subside.

In this process nitrogen in *organic* combination is converted successively into sodium cyanid, sodium ferrocyanid and ferric ferrocyanid ("Prussian blue"). See test **21**.

54. Sulfur. Into a *dry* test tube place a dry piece of metallic sodium the size of a pea and about twice as much dry egg albumen. From this point repeat process **53** until the filtrate containing the soluble products is obtained. In this process *organic* sulfur is converted into sodium sulfid and the sulfid is contained in the filtrate. The presence of sulfid in the filtrate may be determined in various ways as follows (see also tests **59–60**):

55. *Sodium nitroprussid test.* A few drops of sodium nitroprussid solution produce violet coloration of liquids containing alkali sulfids. (**L**: 181.) The nature of the colored product is unknown.

56. *Silver test.* Place a few drops of the solution (**54**) on a silver coin. A black stain indicates sulfid. Equation:

$$2Ag + Na_2S + 2H_2O = \underset{\substack{\text{Silver sulfid}\\(\text{black})}}{Ag_2S} + 2NaOH + H_2$$

57. In some organic compounds sulfur is *firmly* combined in the molecule, in some it is *loosely* united, in others it exists in both conditions of combination.

58. *Tests for loosely united organic sulfur.* Place horn shavings in fairly concentrated sodium hydroxid solution (10 per cent.). Boil the mixture several minutes. In this process *loosely* combined *organic* sulfur is converted to sulfid. As a rule only a part of the total sulfur can be converted to sulfid. Test the filtrate with the following reagents:

59. Lead acetate. Add the reagent drop by drop. Equation (**47**):

$$Na_2S + Pb(C_2H_3O_2)_2 = \underset{\substack{\text{Lead sulfid}\\(\text{black})}}{PbS} + 2NaC_2H_3O_2$$

60. Evaporate a portion nearly to dryness. Acidify with nitric acid and test the gaseous product with wet lead acetate paper (**59**).

61. Apply tests **55** and **56**.

62. *Test for firmly united organic sulfur.* Fuse a *small* quantity

of dry egg albumen with solid potassium hydroxid and potassium nitrate in a porcelain crucible. Continue the fusion until the mass is practically white. Cool. Place the cold crucible and contents in a small beaker with a little water and boil to effect solution. Acidify with hydrochloric acid. The acid may be added before solution is complete in order to hasten the process. Filter. Add barium chlorid solution (42).

In this process *all* the *organic* sulfur is oxidized and soluble alkali sulfate results.*

63. Phosphorus. Dry a small piece of liver in a watch glass on a water bath. Subject the dry mass to process 53 down to the point of acidifying with hydrochloric acid. In this case acidify with *nitric acid* the solution containing the products of the fusion and apply to it test 16 for phosphate.

In this process the *organic* phosphorus is oxidized and soluble alkali phosphate results.

64. Iron. Dry a small amount of blood in a crucible on a water bath. Add a few drops of nitric acid and ignite *cautiously*, until the mass is charred. Add nitric acid occasionally and repeat the ignition process until incineration is complete. Avoid excessive heating. See footnote, page 8. Warm the ash with hydrochloric acid and to this solution apply test 21.

In this process the *organic* iron is oxidized, and converted by hydrochlorid acid to ferric chlorid.

* In quantitative determinations it is usually found that the *sulfid* sulfur obtainable by process 58 is much less in quantity than the *sulfate* sulfur obtainable by process 62. The added quantity obtainable by the latter process is the *firmly* combined sulfur. A vigorous oxidative process is required to remove the firmly combined sulfur from its molecular position.

CHAPTER II.

FATS.

A. Introductory Notes on Some Solvents of General Value in Biochemical Experiments.

65. Kinds. In our study of the properties of biological organic compounds we shall have occasion repeatedly to ascertain the soluble qualities of the substances under examination. In order to compare the biological substances with each other from the standpoint of solubility, it will be desirable to determine the effects of the *same solvents* on the important compounds. As a rule we shall use two series of solvents, which we may designate as *biological* solvents (A) and *special* solvents (B) and which consist of the liquids named below:

A. *Biological solvents:* Water, saliva, gastric juice, pancreatic juice, blood serum, urine.

B. *Special solvents:* Sodium chlorid (5 %), hydrochloric acid (0.2 %), sodium carbonate (0.5%), concentrated hydrochloric acid (39 %), potassium hydroxid (10 %), alcohol (95 %), ether.

In special cases additional solvents, such as bile and chloroform, will be employed.

Water and urine will be supplied in their natural conditions. Common drinking water will be used. Its very slight proportionate content of dissolved matters is without significance in the tests in which it will be employed.

Saliva, gastric juice, pancreatic juice and serum cannot be obtained satisfactorily in sufficient quantities for the many tests that will be made, but there will be available various *artificially prepared* liquids that will closely resemble the corresponding natural products and will manifest exactly their solvent effects (69–78).*

66. Reactions of the natural biological solvents. — Of the

* When only small quantities of *saliva* are needed the filtered natural secretion will be obtained and employed by each student. Normal *serum* will be furnished when only small quantities are required. In particularly important connections solubilities will be *demonstrated* with the *natural* solvents.

biological solvents water is *neutral.* Saliva, pancreatic juice and serum are slightly *alkaline,* by reason of the presence of salts, such as phosphates and carbonates, that undergo hydrolytic dissociation (P : 295). Gastric juice is quite strongly acid because of its content of *free hydrochloric acid* and urine is usually slightly acid because of the preponderance of *acid reacting* salts, chiefly acid phosphates (P : 295).

67. The reactions of these liquids have been stated in terms of their effects on litmus, an indicator that is commonly employed, but which is unsatisfactory in many respects. It is so important for us to clearly understand how much and how little the terms "acidity" and "alkalinity" mean, as they are commonly used in connection with biological liquids, that I shall quote a very satisfactory statement of facts in this connection from an important paper which appeared while the proofs of this volume were being corrected.* Although the following statement was made in reference to urine, it applies equally well to biological liquids in general and expresses the opinion regarding them in this connection that has been rapidly gaining acceptance for several years:

"A large number of methods have been published for determining and estimating the reaction of the urine. . . . The figures vary enormously according to the method and the indicator used for the purpose.

"In addition to the experimental difficulties introduced by the fact that the urine is itself colored, and hence interferes with the delicacy of the reaction to colored indicators, there is the more important fact that the reaction of the urine is never due to free acid or to free alkali, but to a varying mixture of salts such as the primary and secondary phosphates of the alkalies. In such a mixture the urine reacts entirely differently to different indicators. Thus the same sample of normal urine is *acid* to a sensitive indicator to weak acids such as phenol-phthalein and *alkaline* to a stable indicator such as methyl-orange or di-methyl-amido-azo-benzol. Also if the titration figures to three such indicators as phenol-phthalein,

* Edward S. Edie and Edward Whitley. A method for determining the total daily gain or loss of fixed alkali and for estimating the daily output of organic acids in the urine, with applications in the case of *Diabetes mellitus.* The Bio-Chemical Journal, January, 1906 ; vol. I, pages 11, 12 and 13.

litmus, and 'di-methyl' be taken, it will be found that there is a high acidity with the phenol-phthalein, a much lower acidity with the litmus, and a high alkalinity with the di-methyl indicator. When similar titrations are undertaken in the case of an artificial mixture of the primary (NaH_2PO_4) and secondary (Na_2HPO_4) phosphates of sodium in water to which such phosphates have been added in known amount and ratio, the reason underlying the differences in the titration values becomes at once apparent. The neutral point for phenol-phthalein lies at the point where the kations and anions are so distributed as to correspond to Na_2HPO_4, while for di-methyl (or methyl orange) the neutral point corresponds to NaH_2PO_4, and for litmus the neutral point lies somewhere intermediate between these two values. It is clear from this statement that it is futile to regard any one indicator as the arbiter of neutrality, and to consider a solution as being neutral because it is neutral to phenol-phthalein when it is alkaline to litmus and 'di-methyl,' or when it is neutral to litmus or lacmoid, and acid to phenol-phthalein and alkaline to di-methyl at the same time.

"*The only true definition of neutrality would be the point at which the concentrations of hydrogen and hydroxyl ions are equal, and no colored indicator satisfies this condition but indicates neutrality at a point where there is a given ratio other than equality between the acidic and basic ions. The value of this ratio depends on the ease with which the colored indicator, and its ions, associate or dissociate in solution.*

"The proper method of determining reaction ought, therefore, to be some method of determination of the concentration of hydrogen or hydroxyl ions which would indicate where these two concentrations were equal.

"In the case of a solution of the mixed phosphates, such as the urine, all physical methods for determination of the ionic concentrations fail, however, in accuracy on account of the very slow variation of the concentration in the two ions around the neutral point. In the case of free acid or free alkali, the smallest addition of acid or alkali to the solution in the neighborhood of the neutral point causes an immense swing in the ratio of the two ions which is at once obvious in the gas battery; whereas, in the case of a solution containing phosphates, the degree of dissociation is low,

and an addition of acid or alkali causes not a great swing in the ratio of hydrogen or hydroxyl ions but a decomposition of phosphate in either direction, and the establishment of a new equilibrium in which the ratio of the two ions may not be widely different from the former.

"On this account a solution such as the urine, or any of the body fluids in general, behaves as a controlling agent or as a neutralizing agent for either acid or alkali, and prevents large variations in either hydrogen or hydroxyl ion concentration.

"The importance of such a regulating mechanism to the organism, the cells of which are so sensitive to such variations, is too obvious to need elaboration."

68. Digestive powers of the natural biological solvents. Saliva, gastric juice and pancreatic juice are dilute aqueous solutions and each contains *enzymes* that transform *chemically* certain organic substances. Saliva is especially active in transforming (digesting) complex carbohydrates, like starch, into simpler carbohydrates like maltose. Gastric juice vigorously converts (digests) complex proteins, like globulin, into simpler proteins like globuloses. Pancreatic juice accomplishes all in a digestive way that both saliva and gastric juice effect on the two classes of substances indicated, and, besides, it hydrates fats into glycerol and fatty acids (**82**).

Water is free from enzymes. Serum and urine do not manifest digestive effects with sufficient distinctness to require attention in this regard. Serum is a dilute alkaline aqueous solution containing especially proteins and saline matter. Urine is, in the main, a dilute aqueous saline solution, containing especially chlorids, phosphates and sulfates, and several organic substances, among which urea (**L : 251**) is the most conspicuous.

General composition of the natural biological solvents. 69. *Saliva* (*mixed*) is a very slightly alkaline solution (**67**) containing about 0.3 per cent. of protein and other organic matter; also approximately 0.2 per cent. of inorganic compounds, consisting *chiefly* of phosphate, carbonate, chlorid and sulfate of potassium and sodium. It contains traces of enzymes, chief of which is ptyalin, which hydrates polysaccharid to disaccharid. The alkalinity is usually not greater than that of a 0.15 per cent. to 0.2 per cent. solution of sodium carbonate.

The composition of the *artificial* saliva prepared for use in the tests (65) is indicated in section 75.

70. *Gastric juice* is a decidedly acid solution containing about 0.3 per cent. of protein and other organic matters and approximately 0.4 per cent. of inorganic substances. Of these, chlorids of sodium and potassium, and hydrochloric acid, are the most conspicuous. It contains slight quantities of enzymes, chief of which is pepsin, which hydrates complex proteins like albumin to simpler proteins like peptone. The acidity is commonly equal to that of a 0.2 per cent. solution of hydrochloric acid.

The composition of the *artificial* gastric juice prepared for use in the tests (65) is indicated in section 76.

71. *Pancreatic juice* is a slightly alkaline liquid containing about 10 per cent. of solids, of which 9 per cent. consists of organic matter, chiefly proteins. Alkali chlorids, phosphates and carbonates are the chief inorganic constituents. It contains enzymes similar to those in saliva and gastric juice and also lipase, a *fat-splitting* enzyme, in very active proportion. The alkalinity is approximately equal to that of a 0.25 per cent. solution of sodium carbonate.

The composition of the *artificial* pancreatic juice prepared for use in the tests (65) is indicated in section 77.

72. *Serum* is a slightly alkaline liquid containing about 9 per cent. of solid matter, of which about 1 per cent. consists of inorganic substances. The organic matter consists chiefly of proteins and "extractives." The inorganic matter is mainly sodium chlorid, together with slight proportions of the usual salts present in biological liquids. The alkalinity of the serum is not greater than that of a 0.25 per cent. solution of sodium carbonate.

The composition of the *artificial* serum for use in the tests (65) is indicated in section 78.

73. *Urine* is a dilute saline solution that varies considerably in reaction but which is more frequently acid than alkaline. The acidity is probably never greater than that of a 0.1 per cent. solution of hydrochloric acid. The urine usually contains about 3 to 5 per cent. of dissolved substances, although the proportion of soluble matter varies considerably. The reaction is due to hydrolytically dissociated salts, such as phosphates (67). The proportions of total organic and total inorganic matters are about the same. The chief

organic substance is urea, which amounts to very much more than all the other *organic* substances combined. Sodium chlorid often predominates quantitatively over all the other *inorganic* substances combined.

The general composition of *normal* urine is indicated on the opposite page.

74. Approximate composition of the artificially prepared biological liquids to be used in the tests of solubilities. In the artificial preparation of the several "biological" solvents already referred to (65) no attempt has been made to represent all the known constituents of the natural products. It has been considered sufficient for our purposes to make up *aqueous* solutions containing constituents approximately identical in quality and quantity with those occurring in largest proportions in each liquid under natural conditions, and to make composite solutions that may be relied upon to show essentially the same solvent effects as the corresponding natural solutions. It should be understood also that even under perfectly normal conditions the *natural* products vary considerably in composition, the urine especially. Our artificial products will show us the effects that are usually manifested by the natural solutions. Their composition is given below.

75. *Artificial saliva.*

	Per cent.
Albumin	0.30
Diastase (commercial)	0.10
K_2HPO_4	0.10
KCl	0.05
$CaCl_2$	0.03
Na_2SO_4	0.03
$NaHCO_3$	0.02
KSCN	0.01

76. *Artificial gastric juice.*

	Per cent.
Albumin	0.30
HCl	0.20
Pepsin (commercial)	0.10
NaCl	0.10
CaCl	0.05
KH_2PO_4	0.05

77. *Artificial pancreatic juice.*

	Per cent.
Albumin	8.0
NaCl	0.6
$NaHCO_3$	0.2
K_2HPO_4	0.2
Pancreatin (commercial)	0.2
Diastase (commercial)	0.2
Lipase (slightly alkaline glycerol extract of pancreas)	0.2

78. *Artificial blood serum.*

	Per cent.
Albumin	8.00
NaCl	0.70
Meat extract (commercial)	0.10
KCl	0.08
Na_2HPO_4	0.04
$NaHCO_3$	0.02
$CaCl_2$	0.02
$MgSO_4$	0.01

79. *Urine* (*normal*).

(Figures for daily eliminations—periods of 24 hours.)

	Grams.
Water	1,200–1,700
Solids	60–70

Inorganic solids: 25–35 grams.	Grams.	*Organic solids:* 25–35 grams.	Grams.
NaCl	10–20	Urea	20–30
Na, K, NH_4, Ca, Mg ... SO_4, PO_4, CO_3	10–15	Urate, Creatinin, Hippurate, etc.	3–5

B. DEMONSTRATION OF FATTY PRODUCTS.

80. Samples of typical fats, oils, waxes, lecithin, fatty acids, soaps and glycerol.

C. CHEMICAL CONSTITUTION OF FATS (0 : 163).

81. Fats are glyceryl esters of fatty acids.* They occur in both plants and animals, frequently in large proportions. Their constituent radicals are indicated by the following equation:

$$\begin{array}{l} C_{17}H_{35}COO \\ C_{17}H_{35}COO \\ C_{17}H_{35}COO \end{array} \begin{array}{|l} H \quad HO \\ H + HO \\ H \quad HO \end{array} \begin{array}{l} CH_2 \\ CH \\ CH_2 \end{array} = \begin{array}{l} C_{17}H_{35}COO - CH_2 \\ C_{17}H_{35}COO - CH \\ C_{17}H_{35}COO - CH_2 \end{array} + 3H_2O$$

Stearic acid (three molecules) — Glycerol (one molecule) — Tristearin (one molecule)

82. Formulas of typical fats† and the corresponding acids that may be derived from them by direct cleavage (saponification, 119–126).

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*A few fats contain radicals of oxy-fatty acids, such as monoxy-stearic acid, and also of acids that are not members of the common fatty acid series, as in the case of triolein (0 : 163), which contains the residue of oleic acid (82) the eighteenth member of the acrylic acid series. The members of the acrylic acid series are *unsaturated* fatty acids.

†The fats are triglycerids. The more common fats are *simple* triglycerids and consist of glyceryl combined with *three* fatty acid residues of the *same kind*. Thus, tripalmitin contains three palmityl radicals. But most animal and vegetable fatty deposits also contain *mixed* triglycerids, in one type of which only *two* of the acid radicals are the *same* and in the other type the *three acid radicals are different*.

Fats:

$C_3H_7COO-CH_2$	$C_{15}H_{31}COO-CH_2$	$C_{17}H_{35}COO-CH_2$	$C_{17}H_{33}COO-CH_2$
$C_3H_7COO-CH$	$C_{15}H_{31}COO-CH$	$C_{17}H_{35}COO-CH$	$C_{17}H_{33}COO-CH$
$C_3H_7COO-CH_2$	$C_{15}H_{31}COO-CH_2$	$C_{17}H_{35}COO-CH_2$	$C_{17}H_{33}COO-CH_2$
($C_{15}H_{26}O_6$)	($C_{51}H_{98}O_6$)	($C_{57}H_{110}O_6$)	($C_{57}H_{104}O_6$)
Tributyrin	Tripalmitin	Tristearin	Triolein

Fatty acids:

C_3H_7COOH	$C_{15}H_{31}COOH$	$C_{17}H_{35}COOH$	$C_{17}H_{33}COOH$
Butyric acid	Palmitic acid	Stearic acid	Oleic acid

The *unsaturated* character of oleic acid and its close relationship to palmitic acid and to stearic acid are shown by the following equations, which express *oxidation* effects of fusion with potassium hydroxid, and a *reduction* effect of treatment with phosphorus and iodin:

Oxidation: $C_{17}H_{33}COOH + 2KOH = C_{15}H_{31}COOK + CH_3COOK + H_2$
(Oleic acid; Potassium palmitate; Potassium acetate)

Reduction: $C_{17}H_{33}COOH + H_2 = C_{17}H_{35}COOH$
(Stearic acid)

D. General Characters of Common Crude Animal and Vegetable Fats.*

Typical fats for use in the tests to be described. 83. *Kinds.* In experiments 86–161 use samples of one or more of the following common *crude* animal and vegetable fatty products: tallows (beef and mutton), lard, butter, olive oil and myrtle wax.

84. *Nature.* Each of these *crude* fatty materials consists of a *mixture of fats*, and also contains water together with slight quantities of unimportant inorganic and organic matters. Myrtle wax is

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* Fat is a term commonly applied to any mass of fat or fats, pure or crude, such as butter. *A fat* is a particular chemical substance of definite chemical and physical properties, such as tripalmitin (82).

Some fats and waxes are physically very much alike. Fats are esters of a *particular tri-hydric alcohol* of relatively *low* molecular weight, *i. e.*, glycerol, whereas waxes are esters of *various mono-hydric* alcohols of relatively *high* molecular weight, *e. g.*, cetyl palmitate (in spermaceti), $C_{15}H_{31}COO-C_{16}H_{33}$. Wax, like fat, is a crude mixture (0: 162–163).

The terms wax and fat are frequently used interchangeably.

Oils are of three general kinds: (1) *Fatty or fixed oils*, such as olive oil; (2) *essential or volatile oils*, such as oil of turpentine; (3) *mineral oils*, such as kerosene. Fatty oils are of both animal and vegetable origin, and are the only oils that contain true fats. Fatty oil is physically unlike fat in being liquid at room temperature. Qualitatively fats and fatty oils are similar (0: 162).

somewhat exceptional (135). The impurities referred to exert no appreciable influences in the tests to be employed.

85. *Preparation.* Each kind of *animal* fat here referred to is liquid at body temperature but is solid at room temperature. For the preparation of the first three varieties the fatty tissue of the animal is finely divided and heated, when the fat may be expressed in molten condition. It quickly solidifies.

Butter is made from milk by a churning process that brings the suspended fat globules together in buttery masses of irregular size which may be easily pressed together.

Olive oil is obtained from the fruit of the common olive, chiefly by simple expression.

The berries of the wax-myrtle (*Myrica cerifera*) are coated with a fatty layer which is commonly called "myrtle wax," or "myrica tallow." The wax-myrtle and its fruit are also designated by the term bayberry and myrtle wax is, therefore, frequently called "bayberry tallow." *

E. ELEMENTARY COMPOSITION OF FATS.

86. **True fats consist solely of carbon, hydrogen and oxygen.** Apply tests (50–51) to a sample of one of the fatty products. Prove the absence of nitrogen, sulfur, phosphorus and iron, tests 53, 62, 63, 64.

F. PHYSICAL PROPERTIES OF FATS.

87. **Fats are non-volatile** (footnote, page 30). Notice the greasy stains made on paper by soft fats, such as lard and olive oil. Place the spotted paper on a watch glass and heat without burning.

88. **Pure fats are substances of neutral reaction.** — Test with litmus the reaction of *fresh* butter and *fresh* olive oil.

89. **Rancid fat is acid in reaction.** — Apply test 88 to *old* butter and *old* olive oil. The acid in rancid fat results from hydrolysis of fat molecules (especially through the agency of bacteria), as shown by the typical equation on the next page :

* The fruit of the common bay tree or laurel (*Laurus nobilis*) is also termed bayberry. (Bay rum is obtained by distilling with rum the leaves of *Pimenta acris*, one of the members of the myrtle family, or by mixing with alcohol, water and acetio ether, the volatile oil obtained from the leaves by distillation.)

$$\begin{array}{l} C_3H_7COO{-}CH_2 \\ | \\ C_3H_7COO{-}CH \\ | \\ C_3H_7COO{-}CH_2 \\ \text{Tributyrin} \end{array} + 3HOH = 3C_3H_7COOH + \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \\ \text{Glycerol} \end{array}$$

Butyric acid

90. **Solubility.** Test the solubility of one of the fats in all the solvents referred to in section 65.

91. Pour upon a piece of filter paper a drop of any of the true solutions obtained, *e. g.*, the ethereal solution (90). Notice the greasy stain (87) that is produced by evaporation of the solvent.

92. Allow the true solutions (90) to evaporate spontaneously in test tubes. Examine under the microscope the deposits produced. See experiment 101.

93. **Emulsion.** Shake vigorously in a test tube a mixture of about 5 c.c. of water and 2 or 3 drops of fresh perfectly *neutral* olive oil (89). Note the time at which the experiment was begun, and put the tube in a water bath at about 40° C. Ten minutes after the beginning of the experiment examine the liquid under the microscope. Acidify with hydrochloric acid.

94. Repeat experiment 93 but use 5 c.c. of a soap solution instead of water. After microscopic examination, acidify with hydrochloric acid and compare with the result in test 93.

95. Repeat experiment 93 but use 5 c.c. of 0.5 per cent. sodium carbonate or hydroxid solution instead of water. After microscopic examination of the mixture add a drop of oleic acid. Shake the mixture. Repeat the latter part of experiment 94.

96. Examine milk under the microscope and compare with the microscopic observations in experiments 93–95. Acidify the milk as in experiments 93–95.

97. Let a drop of perfectly *neutral* olive oil or melted *fresh* butter fall gently upon the surface of 0.25 per cent. sodium carbonate solution contained in a watch glass. Notice that the film of oil on the surface remains clear. Compare with the results of experiments 98 and 99.

98. Add 2 drops of oleic acid to 5 c.c. of *neutral* olive oil or melted *fresh* butter in a *dry* test tube. Shake vigorously. With a drop of this solution repeat the preceding experiment (97) in another watch glass. Notice the diffusion currents shown by the amœboid movement of the rancid oil. Observe also the spontane-

ous emulsion of the fatty material. Compare with the results of experiment 97.

99. Treat the remainder of the rancid oil prepared in the preceding experiment (98) with 10 more drops of oleic acid. Shake vigorously. Repeat experiment 98 with a drop of this relatively very acid oil. Notice that the drop of oil becomes white and opaque, but that neither the general amœboid movement nor emulsion occurs.

The presence of a large proportion of oleic acid in the oil results in the production of an opaque soapy *coating* on the oil. The caked soapy coating is relatively insoluble, and mechanically prevents the formation of an emulsion as well as the disintegration of the main mass of the oil.

100. Crystallinity. Examine various samples of fat under the microscope.

101. Heat a small quantity of fat with just enough alcohol to dissolve it. Let the mixture cool. Examine the deposit. See experiment 92.

Demonstrations. 102. Influence of albuminous compounds and other substances on emulsion. 103. Preparation of a pure fat. 104. Specific gravity of fats. 105. Effects of lowering the temperature of fatty oils. 106. Effects of raising the temperature of solid fats and waxes. 107. Inflammability of fats. 108. Decomposition of fats by destructive distillation, with an examination of the products. 109. Apparatus for determining the melting point of a substance.

110. Determination of melting point. — Determine by the method shown in the demonstration (109), the melting point of any of the solid fats.

111. Table showing the melting points of typical pure fats and of the corresponding acids that may be derived from them by direct cleavage (saponification, 119–126).

	Formula.		*Melting point.* °C.	
	Fat.	Fatty acid.	Fat.	Fatty acid.
Tributyrin	$C_{15}H_{26}O_6$	n-C_3H_7COOH	?	— 8
Tripalmitin	$C_{51}H_{98}O_6$	$C_{15}H_{31}COOH$	50	63
Tristearin	$C_{57}H_{110}O_6$	$C_{17}H_{35}COOH$	57	70
Triolein	$C_{57}H_{104}O_6$	$C_{17}H_{33}COOH$	— 6	14

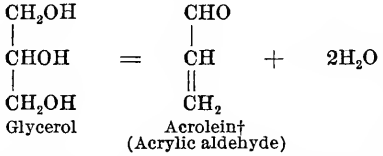
G. CHEMICAL TESTS.

112. Acrolein test. — Heat a small quantity of fat in a *dry* test tube. Pungent fumes of acrolein * will be eliminated. Make a few drops of silver nitrate solution, on a watch glass, alkaline with a drop of ammonium hydroxid. Note the reducing effect of the acrolein fumes on some of this solution in a strip of filter paper suspended directly over the mouth of the test tube.

The characteristic odor of burning fat is partly due to the acrolein that is formed in the process. The maximum production of acrolein requires the presence of a special dehydrating agent. Try the effect of such an agent in the next test.

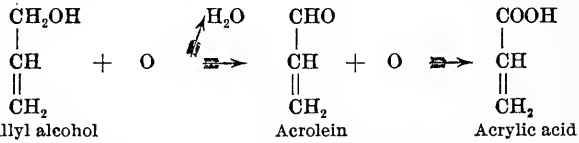
113. Repeat the preceding test, but before heating the fat mix with it about twice its quantity of *dry* potassium hydrogen sulfate, which effects dehydration.

Under the conditions of the acrolein test fat is decomposed into glycerol and other products. The glycerol is dehydrated into acrolein. See test **112**. Equation:



* The name is derived from *acer*, sharp, and *oleum*, oil.

† Acrolein is the aldehyde of allyl alcohol. The corresponding acid is acrylic acid (81). Both compounds are ethylene ($H_2C=CH_2$) or olefin derivatives and are typical *unsaturated* compounds (82). Their relations to each other are indicated by the following formulas:



These three products are the first members of corresponding homologous series. Oleic acid (81) is the eighteenth member of the acrylic acid series. The reducing action shown by acrolein (112) might be inferred from its aldehyde character (L : 226).

In this connection it may be noted that *unsaturated* compounds, such as oleic acid, tend under favorable conditions to undergo conversion into saturated substances, *i. e.*, double bonds between carbon atoms become single bonds (82). In such a conversion, the substances may exert reducing action. Thus, fat containing a radical of any member of the acrylic acid series, such as the radical of oleic

All fats, glycerol and glyceryl-containing substances, such as lecithin (0 : 164), respond to the acrolein test. Allyl alcohol may be readily oxidized to acrolein, its aldehyde (see footnote, page 34).

Demonstrations. 114. Osmic acid and Sudan III tests for fat applied to olein, palmitin, stearin and crude fats. 115. Affinity of fat for iodin. 116. Methods and apparatus for the quantitative determination of fat.

117. Percentage amounts of fat (ether-soluble matter) obtained from some mammalian parts.*

The figures in the subjoined table represent general average contents of fatty matters in the materials named:

Sweat	0.001	Bone (shaft)	1.4
Vitreous humour	0.002	Bile	1.4
Saliva	0.02	Crystalline lens	2.0
Lymph	0.05	Liver	2.4
Synovia	0.06	Muscle	3.3
Liquor amnii	0.06	Hair	4.2
Chyle	0.2	Milk	4.3
Mucus	0.3	Brain	8.0
Blood	0.4	Nerves	22.1
Ligament and tendon	1.1	Adipose tissue	94.6
Cartilage	1.3	Bone Marrow	96.0

The characters of the fatty matters in the parts indicated above will be considered during the study of the tissues, secretions, etc.

H. SAPONIFICATION (0 : 147).

Demonstrations. 118. Various soaps and their properties. 119. Preparation of lead oleate ("lead plaster"). 120. Saponification in distilled water. 121. Saponification in dilute acid. 122. Saponification through the agency of bacteria. 123. Saponification by lipase. 124. Separation of fat from soaps and fatty acids. 125. Effects of fatty acids on carbonates.

126. Saponification of olive oil. To about 10 c.c. of olive oil or the same bulk of any fat in a casserole add 30 c.c. of sodium hydroxid solution (10 per cent.). Boil the mixture until it appears

---

acid (oleyl), blackens osmic acid (demonstration 114) by reducing the latter to a lower oxid (black). Fats like tripalmitin and tristearin, which contain only radicals of *saturated* compounds, do not blacken osmic acid.

*The proportion of fat in some of the above-named parts varies greatly.

to be homogeneous and no oil separates on transferring several drops of it to water in a test tube. Fifteen minutes or more may be required to complete the treatment. Add water occasionally as evaporation goes on in order to maintain approximately the original volume. In this process the fat is completely saponified. Keep the mixture (**127–134**). Typical equation:

$$\begin{array}{l} C_{17}H_{33}COO{-}CH_2 \\ \quad\quad | \\ C_{17}H_{33}COO{-}CH \\ \quad\quad | \\ C_{17}H_{33}COO{-}CH_2 \\ \text{Triolein} \end{array} + 3NaOH = \underset{\text{Sodium oleate (soap)}}{3C_{17}H_{33}COONa} + \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \\ \text{Glycerol} \end{array}$$

127. Frothiness of the soap solution (137). Notice the frothiness of a sample of the soap solution just prepared (**126**). Compare with the frothiness of a solution of a piece of common soap.

128. Emulsifying power of the soap solution (149). Add to a sample of the soap solution prepared in process **126**, a few drops of olive oil. Compare the emulsion effects with those obtained in experiments **93–99**.

129. Process of "salting out" the soap. To a sample of the soap solution (**126**) add small quantities of finely powdered sodium chlorid and stir thoroughly after each addition. Notice the precipitate that rises to the surface of the mixture. Filter the mixture. Make a watery solution of the precipitate and repeat with it experiments **127** and **128**.

This precipitation method is commonly employed in the process of manufacturing solid soaps. The excess of sodium ions in the solution that is introduced by the addition of the sodium chlorid, decreases the solubility of the sodium soaps present in the solution until they are precipitated. The change is only a physical one.

130. Conversion of the sodium soaps into soaps of other metals. To a sample of the soap solution (**126**) add calcium chlorid solution. An insoluble calcium soap is formed. Calcium soaps are formed when soluble soaps are dissolved in "hard" water. Typical equation:

$$2C_{17}H_{33}COONa + CaCl_2 = \underset{\text{Calcium oleate}}{(C_{17}H_{33}COO)_2Ca} + 2NaCl$$

131. Repeat experiment **130** with solutions of salts of heavy metals, such as lead acetate, ferric chlorid and copper sulfate.

132. Decomposition of sodium soap into fatty acid and common salt. To the remainder of the soap solution prepared in process **126** add sufficient hydrochloric acid to make the mixture acid. Fatty acids rise to the top of the mixture. Typical equation:

$$C_{17}H_{33}COONa + HCl = C_{17}H_{33}COOH + NaCl$$

133. Conversion of fatty acid into soap (148). Transfer the mixture (**132**) to a *wet* filter, which will retain the fatty acids. Carefully neutralize the filtrate with sodium hydroxid and retain it for use in test **161**. Remove the mixture of fatty acids from the filter. Add to it sodium hydroxid and convert the acids into soaps with the aid of heat.

134. To the solution just prepared apply tests **127** and **128**.

I. PREPARATION OF A SINGLE FATTY ACID IN COMPARATIVELY PURE CONDITION.

135. Myrtle wax (**85**) consists chiefly of palmitic acid. About one fifth of the material is tripalmitin, and approximately four fifths of it is *free* palmitic acid. The material also contains a trivial proportion of lauric acid, $C_{11}H_{23}COOH$, either free or as trilaurin or perhaps in both forms.

Prepare palmitic acid from myrtle wax by the following process **136–142**.

136. Saponification. Place 10 grams of myrtle wax * in 150 c.c. of water contained in a small casserole. Heat the mixture and stir it while the temperature rises. After the fat has been melted, add to the mixture about 50 c.c. of potassium hydroxid solution (10 per cent.) and boil vigorously until saponification is complete, *i. e.*, until the solution fulfills the conditions mentioned in connection with process **126**. Typical equations:

$$\begin{array}{l} C_{15}H_{31}COO{-}CH_2 \\ \quad\quad | \\ C_{15}H_{31}COO{-}CH \\ \quad\quad | \\ C_{15}H_{31}COO{-}CH_2 \end{array} + 3KOH = 3C_{15}H_{31}COOK + \begin{array}{l} CH_2OH \\ \quad | \\ CHOH \\ \quad | \\ CH_2OH \end{array}$$

$$C_{15}H_{31}COOH + KOH = C_{15}H_{31}COOK + H_2O$$

137. Observe the frothiness and emulsifying power of the soap solution just produced (**127–128**).

* The color of the wax is due to a pigment and not to the fatty matter in it. The fragrant odor, also, is due to non-fatty matter.

138. Liberation of the fatty acid. To the *hot* soapy mixture produced in the process just described * (**136**) add sufficient concentrated hydrochloric acid to acidify the mixture *slightly*. Stir thoroughly after each addition of mineral acid and test with litmus strips the non-oleaginous portion of the mixture. Notice the heavy layer of liquid fatty acid that is finally produced (**132**). After removing the stirring rod from the mixture, pour the latter into a *small* beaker and set the beaker and contents in a water bath containing *cold* water. Cool the mixture *without stirring it*, so as to favor solidification of the palmitic acid in the form of a thin cake. Keep *cold* water in the bath.

139. Solution of the fatty acid. After the palmitic acid has become quite solid lift the cake from the cold subnatant liquid. Keep the liquid for use in test **161**. Spray the cake with water to wash off adherent portions of the slight excess of hydrochloric acid used to separate the palmitic acid from the soap, break the cake into small pieces and transfer the pieces to about 250 c.c. of hot 95 per cent. alcohol in a beaker on a water bath. Heat the alcoholic mixture on the bath, with frequent stirring, until most of the palmitic acid has gone into solution, when the alcohol will be nearly if not wholly *saturated* by it at the prevailing temperature.

140. Crystallization of the fatty acid. Immerse in hot water (in a large beaker) a small beaker whose bottom is covered with a thin layer of *warm* 95 per cent. alcohol. In the latter collect the palmitic acid solution after its passage through a *dry* filter on a *dry* though *warm* funnel. Do not pour on the filter any *oily undissolved palmitic acid* in the alcoholic mixture. Let the alcoholic filtrate cool *undisturbed*. Observe the cumulative formation of a white deposit (palmitic acid) as the temperature falls.

141. Purification of the fatty acid. — Examine under the microscope a sample of the palmitic acid deposited.† Compare with the crystals observed in experiments **100** and **101**. Filter the mixture. Wash the crystals free from adherent acid with cold 95 per cent. alcohol. Notice that a sample of the alcoholic filtrate yields

* If the solution was permitted to cool perceptibly, bring it again to the boiling point and proceed at once with the addition of hydrochloric acid.

† Any lauric acid (135) that may be present in the myrtle wax tends to remain in solution at this point. Lauric acid is comparatively soluble in cold alcohol.

a precipitate when treated with water. The precipitate consists of palmitic acid, which is insoluble in water (145). The remainder of the alcoholic filtrate will be collected for use in other connections.

142. Redissolve the palmitic acid in a small proportion of hot alcohol, recrystallize by cooling the filtered solution, examine under the microscope the new crop of crystals, filter off the product and wash with cold 95 per cent. alcohol for final purification. Dry the washed palmitic acid on a watch glass in an air bath at about 40° C., and test its properties by the methods indicated below.

J. PROPERTIES OF A TYPICAL FATTY ACID (PALMITIC ACID).

143. **Reaction to litmus** (88).

144. **Reaction to phenolphthalein.** — Dissolve some of the palmitic acid in 95 per cent. alcohol. Warm to favor solution.

Into 5 c.c. of water place a few drops of phenolphthalein solution and add to it a drop or two of very dilute sodium carbonate solution in quantity sufficient to induce a permanent red coloration.

To the red solution add the palmitic acid solution drop by drop until acidity is indicated by the discharge of the color.

145. **Solubilities** (65).

146. **Melting point** (110).

147. **Acrolein test** (113).

148. **Conversion into soap.** (133) Melt the remainder of the palmitic acid at a *low* temperature in a small beaker. Add to the oil small quantities of sodium hydroxid solution with repeated stirring and *constant warming*, until the mixture is made *slightly* alkaline in reaction. Pour about half of the liquid into another beaker and let it cool. A hard opaque soap is obtained. To the liquid portion remaining in the original beaker add a small quantity of alcohol, stir thoroughly and let the mixture cool. A hard transparent soap is obtained. The alcohol exerts merely a physical influence.

149. Dissolve some of the soap in water and apply to the solution tests 127 and 128.

K. PROPERTIES OF GLYCEROL.

Demonstrations. 150. Tests of the diffusibility of fats, fatty acids, soaps and glycerol. 151. Preparation of glycerol

on a large scale, and identification of the product. 152. Solidification of glycerol. 151. Distillation of glycerol with steam. 154. Borax bead test for glycerol.

Test small quantities of commercial glycerol as follows:

155. Taste.

156. Reaction to litmus (143).

157. Solubilities (65).

158. Acrolein test (113).

159. **Solvent action on a metallic hydroxid.** Prepare cupric hydroxid by adding several drops of potassium hydroxid solution (10 per cent.) to about 10 c.c. of cupric sulfate solution (2 per cent.). Filter and wash the precipitate with water. Mix on a watch glass a drop of glycerol with a drop of water. On a second watch glass mix a drop of glycerol with a drop of potassium hydroxid solution. Place two drops of water on a third watch class. To the liquid on each watch glass transfer small, approximately equal amounts of the washed cupric hydroxid. Observe the solvent action of the glycerol.*

160. **Dunstan's test.** Add to 5 c.c. of a borax solution (5 per cent.) a quantity of alcoholic solution of phenolphthalein (1 per cent.) sufficient to produce a permanent and distinct red color. Add to the latter drop by drop aqueous solution (10 per cent. or less) of glycerol until the red color is discharged. On boiling the solution, the red color returns. *Excess* of glycerol prevents restoration of the color.

At present this reaction cannot be satisfactorily explained. Ammonium salts behave like glycerol in discharging the color, but they prevent its restoration. The reaction is given by polyhydric alcohols in general (**O**: 84). Among the latter, sugars are conspicuous in biological materials. Glycerol may be separated from the sugars by the distillation method already demonstrated (153).

161. **Detection of glycerol among the products of saponification.** Filter through wet paper the liquids obtained in experiments 133 and 139. Carefully neutralize both filtrates and apply Dunstan's test to each. Demonstration 154:

---

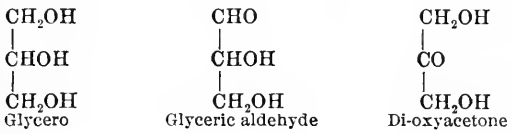
* There is probably a chemical reaction between the copper cation and the glycerol, with the formation of a product similar to an alcoholate (**O**: 81). The color is doubtless due to a complex copper-organic ion (**L**: 226, 252).

Make a larger volume of the copper-glycerol solution and observe that boiling does not decompose it (**L**: 226).

L. CARBOHYDRATE DERIVATIVES OF GLYCEROL.

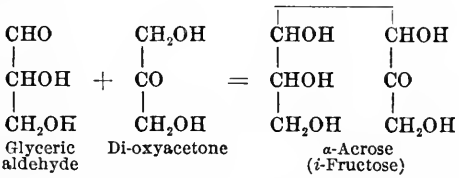
When glycerol is carefully oxidized, *e. g.*, with bromin and sodium hydroxid, a viscous liquid is produced that shows a number of the properties of aldehydes and ketones. The product is called "glycerose" (crude) and contains glyceric aldehyde and dioxyacetone, which are isomeric glyceroses.

The relations of these products to glycerol may be seen at a glance below:

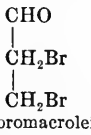


Each of these initial oxidation products of glycerol has many of the properties of the simplest sugars and *each is a carbohydrate.*

It was stated above that crude glycerose, containing glyceric aldehyde and di-oxyacetone, can be prepared by careful oxidation of glycerol with bromin and sodium hydroxid. The latter not only assists in the formation of the oxidation products but it effects a *condensation* of them (**0 : 106**), and a typical sugar, *α-acrose*, results. Equation:

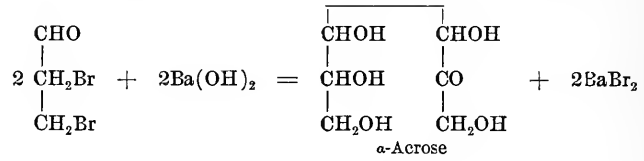


The term acrose was applied to this sugar because the product was originally obtained from acrolein (**113**). When acrolein is properly treated with bromin, two hydroxyl radicals are replaced by bromin atoms and di-bromacrolein results:



Di-bromacrolein yields two isomeric sugars on treatment with

barium hydroxid, one of which is *α*-acrose, as is indicated below:



α-Acrose is a constituent of crude formose (**0 : 108**).

These observations show clearly that *sugar may be made from fat.* Sugars are fermentable. Among the products of fermentation of sugars are *glycerol and fatty acids.* These facts show that *fat may be produced from sugar.*

These matters will be discussed in subsequent chapters (second part) and in the lectures.

BIOCHEMICAL NOTES:

LABORATORY WORK

[SECOND PART]

BY

WILLIAM J. GIES

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PREFACE.

When the required course in physiological chemistry at the College of Physicians and Surgeons was started early in February, 1906, the first part only of this volume was ready for immediate use. The second part of the volume consists of two additional chapters that could be conveniently prepared before the work to which they relate was undertaken.

WILLIAM J. GIES.

LABORATORY OF PHYSIOLOGICAL CHEMISTRY,
COLLEGE OF PHYSICIANS AND SURGEONS,
COLUMBIA UNIVERSITY, March 1, 1906.

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[SECOND PART.]

CHAPTER III.

CARBOHYDRATES.

A. Demonstration of Carbohydrate Products.

163. Samples of typical sugars, dextrins, glycogen, gums, starch, inulin and cellulose.

B. Chemical Constitution of Carbohydrates.

164. General elementary composition and proportions. — Carbohydrates, like the fats, consist of carbon, hydrogen and oxygen. The fats contain relatively small proportions of oxygen and comparatively large proportions of carbon and hydrogen. The carbohydrates, on the other hand, contain relatively small proportions of carbon and hydrogen and comparatively large proportions of oxygen. The carbohydrates represent a more advanced stage of carbon-hydrogen oxidation (188) than the fats, and therefore are as a rule less prone to unite with additional atoms of oxygen. Consequently the carbohydrates possess less potential energy; they yield less heat on combustion. The subjoined table presents results that make these facts quite evident.

	Formula	Percentage Composition			Approximate Atomic Ratios			Heat of Combustion: calories per gram
		C	H	O	C:H	C:O	H:O	
Typical carbohydrate: *glucose.*	$C_6H_{12}O_6$	40.00	6.66	53.34	1:2	1:1	2:1	3763
Typical fat: *tristearin.*	$C_{57}H_{110}O_6$	76.85	12.36	10.79	1:2	9:1	18:1	9530*

The conditions indicated above account for the well-known inflammability of fats (107) and the fact that most carbohydrates, sugars especially, do not take fire so readily (216). Some carbohydrates, however, such as cellulose, which contain the smallest proportions of oxygen and the largest proportions of carbon, burn easily and without sooty flames. The very large proportions of carbon in fats account for the usual sootiness of the flame of burning fat — much of the carbon escapes oxidation.

* Heat of combustion of mutton tallow, which consists largely of tristearin. That of pure tristearin, which has not been determined, must be somewhat higher.

Fats and polysaccharids are the chief carbonaceous reserve materials in organisms. In animals both classes of materials are particularly involved in processes connected with the maintenance of body temperature.

Practically all the carbohydrates contain hydrogen and oxygen in the proportion of two atoms of hydrogen to one of oxygen — the ratio in which these elements occur in water.* This observation led promptly to the selection of the name carbohydrate (*carbon hydrate*) for such carbon compounds.

In harmony with the observation just referred to the general composition of the carbohydrates may be expressed by the formula $C_x(H_2O)_y$, in which x and y represent either the same or different multiples, as is indicated below in connection with the formulas of some common carbohydrates (165):

Glucose, $C_6H_{12}O_6 = C_6(H_2O)_6$, in which $x = 6$ and $y = 6$
Sucrose, $C_{12}H_{22}O_{11} = C_{12}(H_2O)_{11}$, in which $x = 12$ and $y = 11$
Starch, $(C_6H_{10}O_5)_n = [C_6(H_2O)_5]_n$, in which $x = n6$ and $y = n5$

Such formulas mask the more significant intramolecular relationships that are pointed out on page 52.

165. Relative elementary composition and chemical classification of the carbohydrates of greatest biological importance. A general classification of the leading carbohydrates is given in the table on page 49. See the equations in section 169.

166. Relations of the carbohydrates to polyhydric alcohols. All carbohydrates may be regarded as *oxy-derivatives of polyhydric alcohols* (0 : 84).

167. *Monosaccharids.* The *simplest* carbohydrates, or monosaccharids (165), are either hydroxy-aldehydes or hydroxy-ketones (0 : 97), *i. e., aldehyde-alcohols or ketone-alcohols.* The monosaccharids may be converted by *reduction* into their corresponding polyhydric alcohols, just as certain polyhydric alcohols may be *oxidized* to their corresponding sugars (169).

All monosaccharids (and all other carbohydrates) contain two or more hydroxyl groups. In each of the monosaccharids one of the

* There are a number of non-carbohydrate substances, such as acetic acid, $C_2H_4O_2$, and lactic acid, $C_3H_6O_3$, in which the same H : O proportion exists. In a few unimportant carbohydrates, such as rhamnose, $C_6H_{12}O_5$, the ratio H : O is not the same as in water.

(165) Classification of the More Important Carbohydrates.

	Sugars *				Polysaccharids †	
	Monosaccharids (p. 51)		Disaccharids	Trisaccharids	Pentosans ‡	Hexosans ‡
	Pentoses §	Hexoses				
Group formula	$C_5H_{10}O_5$	$C_6H_{12}O_6$	$C_{12}H_{22}O_{11}$	$C_{18}H_{32}O_{16}$	$(C_5H_8O_4)_n$	$(C_6H_{10}O_5)_n$
Empirical analytic group formulas	$C_5(H_2O)_5$ or $C_5H_8O_4 + H_2O$	$C_6(H_2O)_6$ or $C_6H_{10}O_5 + H_2O$	$C_{12}(H_2O)_{11}$ or $2(C_6H_{10}O_5) + H_2O$ or $2(C_6H_{10}O_5, H_2O) - H_2O$	$C_{18}(H_2O)_{16}$ or $3(C_6H_{10}O_5) + H_2O$ or $3(C_6H_{10}O_5, H_2O) - 2H_2O$	$[C_5(H_2O)_4]_n$ or $n(C_5H_8O_4, H_2O) - nH_2O$	$[C_6(H_2O)_5]_n$ or $n(C_6H_{10}O_5, H_2O) - nH_2O$
Names of representative members of the group.	*i*-Arabinose *l*-Xylose	*d*-Glucose *d*-Fructose *d*-Galactose	Sucrose Lactose Maltose Isomaltose	Raffinose, or meletriose	Araban Xylan	Celluloses Starches Glycogens Dextrins Inulin

Synonyms — Starch : amylum.
Cellulose : wood fiber.
Glycogen : animal starch.
d-Glucose : glucose, grape sugar, diabetic sugar, dextrose.
d-Fructose : fructose, fruit sugar, levulose.
Sucrose : cane sugar, saccharose.
Lactose : milk sugar.
Maltose : malt sugar.
Arabinose : pectin sugar.
Xylose : wood sugar.

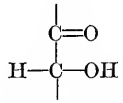
* There are several unimportant tetrasaccharids : $C_{24}H_{42}O_{21}$.

† This term is usually applied to all carbohydrates not included in the mono-, di-, tri- and tetrasaccharid groups. It is sometimes applied to all carbohydrates not included in the monosaccharid group.

‡ Some polysaccharids appear to be composed of both pentose and hexose radicals.

§ The chemical names of the sugars end in *ose*. This suffix is not, however, a characteristic of the chemical names of the sugars, for the name of a typical polysaccharid (cellulose) contains it, polysaccharids are often called polyoses and the same suffix is contained in the names of some common proteins, such as albumose and globulose, which are among the many members of the group of proteoses (256).

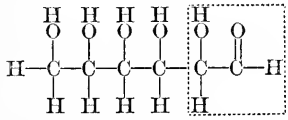
hydroxyl groups is directly connected with a carbon atom that is linked to a (*a*) hydrogen atom, and (*b*) to a carbonyl group; and which is attached by its fourth bond to either a hydrogen or a carbon atom. The characteristic group in every monosaccharid is the following:



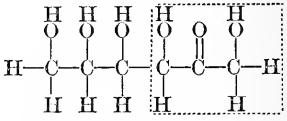
Characteristic monosaccharid group

The monosaccharids of the aldehyde-alcohol type are known as *aldoses*. Those of the ketone-alcohol type are called *ketoses*. In the aldoses the carbonyl group is linked to one hydrogen atom and to one alcohol group. In the ketoses the carbonyl group is held between two alcohol groups.

The following constitutional formulas illustrate these relationships:

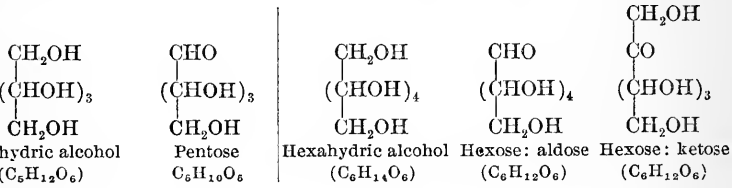


Constitutional formula of a typical aldose
Glucose



Constitutional formula of a typical ketose
Fructose

The constitutional relations between typical polyhydric alcohols and corresponding monosaccharids is shown below:



Pentahydric alcohol ($C_5H_{12}O_6$)	Pentose $C_5H_{10}O_5$	Hexahydric alcohol ($C_6H_{14}O_6$)	Hexose: aldose ($C_6H_{12}O_6$)	Hexose: ketose ($C_6H_{12}O_6$)

The two monosaccharid groups that were named in the above summary, *i. e.*, pentoses and hexoses, are the most important biologically but certain additional monosaccharid groups * are of general chemical interest, especially in connection with the polyhydric relationships of the carbohydrates, as may be seen on the opposite page.

* Each of these special groups consists of laboratory products. None of them occurs naturally. Each group contains aldose and ketose representatives.

Alcohols:

CH_2OH | CH_2OH	CH_2OH | $CHOH$ | CH_2OH	CH_2OH | $(CHOH)_2$ | CH_2OH	CH_2OH | $(CHOH)_5$ | CH_2OH	CH_2OH | $(CHOH)_6$ | CH_2OH	CH_2OH | $(CHOH)_7$ | CH_2OH
Glycol	Glycerol	Erythrol	Glucoheptol	Glucoctol	Glucononol

Sugars:

CHO | CH_2OH	CHO | $CHOH$ | CH_2OH	CHO | $(CHOH)_2$ | CH_2OH	CHO | $(CHOH)_5$ | CH_2OH	CHO | $(CHOH)_6$ | CH_2OH	CHO | $(CHOH)_7$ | CH_2OH
Glycolose	Glycerose	Erythrose	Glucoheptose	Glucoctose	Glucononose
Diose, $C_2H_4O_2$	Triose, $C_3H_6O_3$	Tetrose, $C_4H_8O_4$	Heptose, $C_7H_{14}O_7$	Octose, $C_8H_{16}O_8$	Nonose, $C_9H_{18}O_9$

168. *Di-, tri-, tetra- and polysaccharids.* The more complex carbohydrates, *i. e.*, those not included in the monosaccharid group, are *anhydrids of aldehyde-alcohols or ketone-alcohols* or both, and may be readily converted by hydration into hydroxy-aldehydes or hydroxy-ketones or both, *i. e.*, into the simplest types of sugars, the monosaccharids (**170**). The carbohydrates that are not included in the monosaccharid group may be considered as anhydrids of monosaccharids.

169. General carbohydrate relationships. General carbohydrate relationships are indicated by the following equations in which effects of *oxidation, dehydration, reduction* and *hydration* are shown:

Oxidation (175):

$$\underset{\text{Hexahydric alcohol}}{C_6H_{14}O_6} + O = \underset{\text{Monosaccharid (Hexose)}}{C_6H_{12}O_6} + H_2O$$

$$\underset{\text{Pentahydric alcohol}}{C_5H_{12}O_5} + O = \underset{\text{Monosaccharid (Pentose)}}{C_5H_{10}O_5} + H_2O$$

Dehydration (165):

$$2C_6H_{12}O_6 - H_2O = \underset{\text{Disaccharid}}{C_{12}H_{22}O_{11}}$$

$$nC_6H_{12}O_6 - nH_2O = \underset{\text{Polysaccharid (Hexosan)}}{(C_6H_{10}O_5)_n}$$

$$nC_5H_{10}O_5 - nH_2O = \underset{\text{Polysaccharid (Pentosan)}}{(C_5H_8O_4)_n}$$

Reduction:

$$C_6H_{12}O_6 + H_2 = C_6H_{14}O_6$$

$$C_5H_{10}O_5 + H_2 = C_5H_{12}O_5$$

Hydration (165, 176):

$$(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6$$
$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$$
$$(C_5H_8O_4)_n + nH_2O = nC_5H_{10}O_5$$

170. The various *monosaccharid* derivatives that may be obtained by simple hydration from the important carbohydrates of greater complexity are indicated in the following summary:

Polysaccharids:

Hexosans,

Cellulose * — Glucose (*n*).
Starch — Glucose (*n*).
Glycogen — Glucose (*n*).
Dextrin * — Glucose (*n*).
Inulin — Fructose (*n*).

Pentosans,

Araban — Arabinose (*n*).
Xylan † — Xylose (*n*).

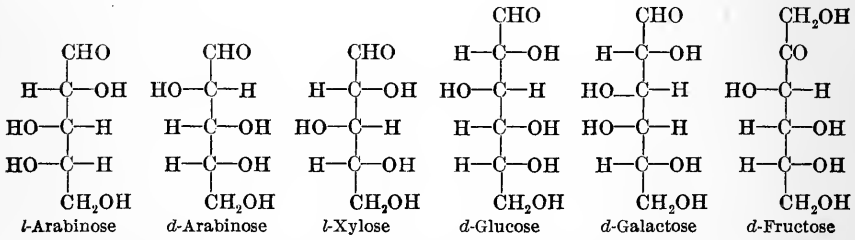
Trisaccharid:

Raffinose — Glucose, fructose and galactose.

Disaccharids:

Sucrose — Glucose and fructose.
Lactose — Glucose and galactose.
Maltose — Glucose (2).
Isomaltose — Glucose (2).

Constitutional formulas of typical carbohydrates. **171.** *Monosaccharids.* The appended formulas indicate the constitution and the *stereochemical relationships* of some of the monosaccharids:

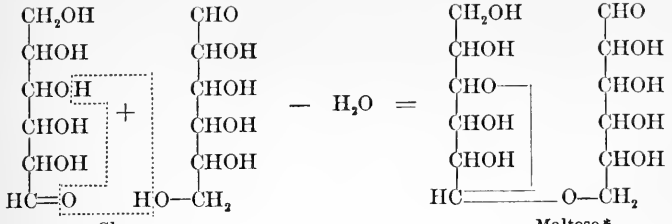


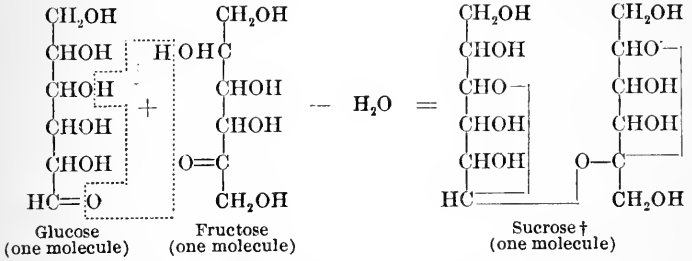
The members of each main group of carbohydrates are *isomers.* The members of each monosaccharid subgroup, such as the aldohexose group (167), have the same *constitutional* formula and are *stereo*-isomers (0 : 28).

172. *Disaccharids.* The constitutional formulas of some disaccharids and the relations of disaccharids to monosaccharids are shown by the equations on the opposite page.

* Some varieties of cellulose and dextrin yield mannose (an isomer of glucose) instead of glucose; also pentoses.

† Some varieties of xylan yield arabinose.

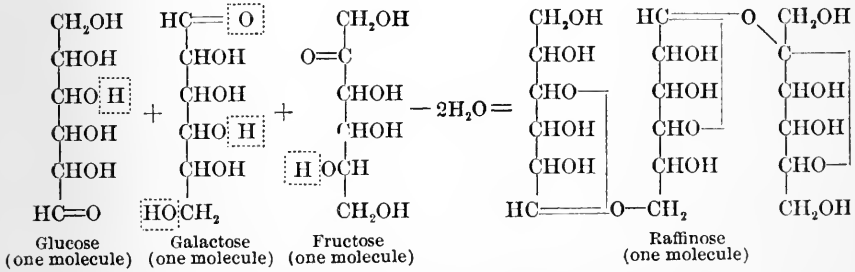




Each of the above formulas for disaccharids may be written empirically as follows :

$$C_6H_{11}O_5—O—C_6H_{11}O_5$$

173. *Trisaccharids.* The constitutional formula of raffinose is indicated below :



The formula for raffinose may also be written as follows :

$$C_6H_{11}O_5—O—C_6H_{10}O_4—O—C_6H_{11}O_5$$

174. *Polysaccharids.* The constitutional formulas of the polysaccharids are unknown. That they are analogous to those of the di-

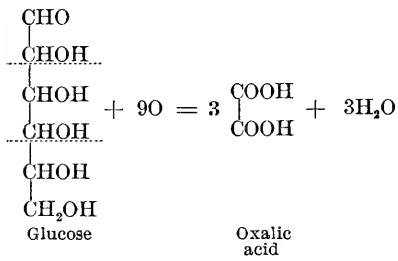
* The formula of lactose (composed of the residues of a molecule of glucose and one of galactose) is essentially the same as that of maltose (173).

† Notice the fact that the sucrose formula is without a free carbonyl group (225).

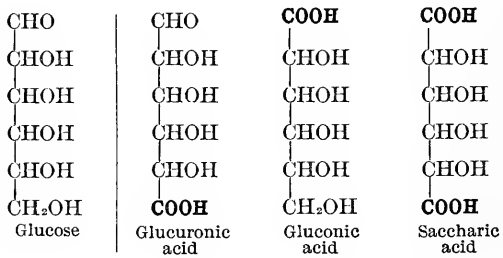
and trisaccharids seems probable. The empirical formula of starch, $(C_6H_{10}O_5)_n$, may be written as follows (**173**):

$$C_6H_{10}O_5—O.....C_6H_{10}O_4—O—C_6H_{10}O_4.....O—C_6H_{10}O_5$$

Decomposition products. **175.** *Products of oxidation* (**169**). The carbohydrates may be readily oxidized to carbon dioxid and water but all of them yield various organic acids upon less vigorous oxidation. Oxalic acid, for example, may be readily produced by profound oxidation (**193**). Equation:



Most of the acids that result from direct oxidation of the carbohydrates are devoid of biological interest. The following formulas represent the most important types of acids that result from relatively *slight* oxidation:

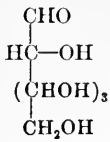


Of the three acids represented by the above formulas glucuronic acid is particularly important. It is a constituent of normal urine.

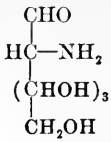
176. *Products of hydration* (**169**), such as formic, acetic, propionic, lactic, butyric acids and other fatty acids, and related substances, are formed from carbohydrates when hydrolysis is carried to the point of decomposition (**0 : 135–140**).

177. *Glucosamin* is an important carbohydrate derivative. It is closely related both to hexoses and to various amino-acids (**0 : 184**)

that may be derived from proteins (250). The relation between glucose and glucosamin is indicated by the following formulas:



Glucose



Glucosamin

C. FORMATION OF CARBOHYDRATES (162).

Laboratory conversion of one carbohydrate into another. 178. *Polysaccharids.* Of the polysaccharids named in the table on page 49, only dextrins can be made in the laboratory. A method for the preparation of dextrin is indicated in section 192.

179. *Sugars.* Both di- and monosaccharids can be obtained from polysaccharids by hydration (169). Monosaccharids can be obtained from the more complex sugars by the same process (169).

Maltose has been made from concentrated solutions of glucose by hydration through the agency of an enzyme called *maltase.* This enzyme is also able to bring about the conversion of maltose into glucose. The hydration process that is induced by maltase is obviously reversible and may be represented thus:

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O \rightleftharpoons \underset{\text{Glucose}}{2C_6H_{12}O_6}$$

Laboratory production of carbohydrates from non-carbohydrates. 180. The monosaccharids have been made in the laboratory from non-carbohydrate materials by several methods. With the exception of maltose (179) none of the higher carbohydrates named on page 49 has been produced synthetically. The transformation of polyhydric alcohols by oxidation into sugars has already been referred to (169).

181. *Synthesis of i-Fructose.* At the close of the preceding chapter (Fats, page 41) attention was drawn to some carbohydrate derivatives of glycerol. Allusion was made to facts showing that carbohydrate may be made from fat.

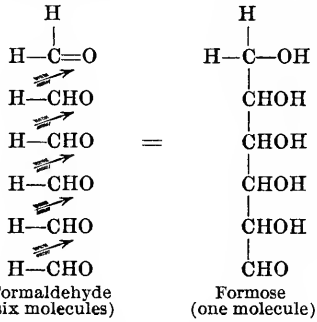
The formulas and equations on page 41 (first part of the volume) explain the derivation of glyceroses (trioses), from glycerol and also the condensation of *i*-fructose (*a*-acrose), from the glyceroses: glyceric

aldehyde and di-oxyacetone. It was stated, in connection with the equation showing this condensation, that *i*-fructose is a constituent of crude formose (**0 : 108**).

182. *Synthesis of crude formose.* Treated with saturated lime water for several days at room temperature, formaldehyde gradually undergoes polymerization (by aldol condensation, **0 : 106**). The product of the polymerization is a sweet syrup, which is called crude formose, and contains formoses and *a*-acrose. Equation :

$$6H—CHO = C_6H_{12}O_6$$

The condensation may be represented graphically as follows :



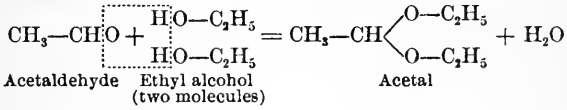
183. Natural formation of carbohydrates from non-carbohydrates. The synthesis of hexoses from formaldehyde (**182**) has an important bearing on the production of carbohydrates from *inorganic* matter in plants.

Carbon dioxid is absorbed from the air into plants. Water is present in large proportions throughout all the green parts of living plants. The chlorophyl (green coloring matter) of plants has the power, under the influence of sunlight, to effect combinations between carbon dioxid and water. It is generally believed that formaldehyde is produced by reduction from the carbon dioxid and water, that the resultant formaldehyde is polymerized into glucose and that the latter is dehydrated into more complex carbohydrates, such as starch. These chemical changes doubtless occur in harmony with the following equations :

$$CO_2 + H_2O = H—CHO + O_2$$
$$6H—CHO = C_6H_{12}O_6 \text{ [182]}$$
$$nC_6H_{12}O_6 — nH_2O = (C_6H_{10}O_5)_n \text{ [169]}$$

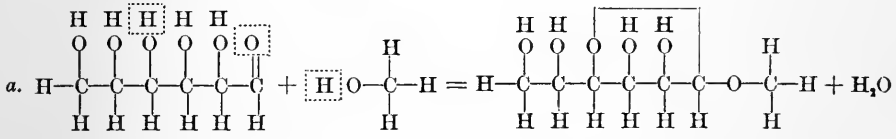
The probability that these deductions are correct is increased by the fact that traces of formaldehyde have been detected in the green parts of plants. It has been shown that in certain sea weeds (Spirogyra), formaldehyde sodium bi-sulfite (**0 : 108**) is decomposed by the living cells, and that the liberated formaldehyde is immediately condensed to sugar and precipitated in the cells as starch.*

184. Glucosids. *Acetals.* Aldehydes unite with alcohols, in the proportion of one molecule of the former to two of the latter, with elimination of water, to form acetals (**0 : 105**). The reaction is induced by mineral acid. Equation :



185. *Synthetic glucosids.* Glucose can be brought into union with alcohols, in a reaction similar to that shown above (**184**), to form a group of substances called glucosids, which are analogous to the natural glucosids. † Typical equations :

a.



b. $C_6H_{12}O_6 + C_3H_5(OH)_3 = C_6H_{11}O_5{-}O{-}C_3H_5(OH)_2 + H_2O$

Glycerol — Glycero-glucosid

In these reactions *only one* alcohol molecule unites with a single aldose molecule. One of the hydroxyl groups of the latter plays

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* These facts suggest that formaldehyde, if produced in plants, as seems to be indicated, is a very *transient* product and under normal conditions never accumulates in sufficient proportion to exert its well-known destructive effects on protoplasm.

† The *natural glucosids* are carbohydrate esters, *i. e.*, "combined" carbohydrates. On hydration with acids or enzymes, or by electrolytic cleavage, monosaccharids are produced from them. As a rule the term glucosid is restricted to plant products of this nature, of which there are many varieties. In a general way the term is sometimes applied to all substances that are not true carbohy-

the part usually taken by the second molecule of alcohol in acetal formations. On hydration of the glucosid the aldose and alcohol are regenerated; thus (**0 : 160**):

$$C_6H_{11}O_5—O—CH_3 + H_2O = C_6H_{12}O_6 + CH_3OH$$

The combination of two monosaccharid molecules with elimination of a molecule of water to form a disaccharid (**172**) is analogous to the production of a glucosid from an alcohol and a monosaccharid.

D. Relationship Between Fat and Carbohydrate.

186. Fats may be converted into carbohydrates and vice versa. Both fats and carbohydrates yield glycerol and fatty acids under favorable conditions of decomposition. At the conclusion of the preceding chapter, on fats, it was stated that carbohydrates may be made from fats. The production of glyceroses and *a*-acrose from glycerol was explained. We have seen how the simplest fatty acid may be converted into carbohydrate (**182**). In our study of metabolism we shall learn that other fatty acids than formic acid appear to be convertible into carbohydrate. Synthesis of fats from fatty acids and glycerol may be effected without difficulty.

187. Relative degrees of oxidation. Fat may arise in organisms from carbohydrates by processes of *reduction* or *dehydration*, and carbohydrates may be formed in organisms from fat by processes of *oxidation* or *hydration*. The general chemical relationship existing between fats and carbohydrates may be understood from a study of typical formulas.

An examination of the formulas of typical fats and carbohydrates shows at a glance the great difference in the degree of oxidation of the two classes of substances (**164**):

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drates, but which, on decomposition, yield monosaccharid molecules. In the latter sense even some proteins, like mucoids (250), are glucosids.

Typical natural glucosids and their initial cleavage products are named below:

$$\underset{\text{Salicin}}{C_{13}H_{18}O_7} + H_2O = \underset{\text{Saligenin}}{C_7H_8O_2} + \underset{\text{Glucose}}{C_6H_{12}O_6}$$

$$\underset{\text{Amygdalin}}{C_{20}H_{27}NO_{11}} + 2H_2O = \underset{\substack{\text{Benzal-}\\\text{dehyde}}}{C_6H_5—CHO} + \underset{\substack{\text{Hydrocy-}\\\text{anic acid}}}{HCN} + 2C_6H_{12}O_6$$

$$\underset{\substack{\text{Sinigrin}\\\text{(Potassium myronate)}}}{C_{10}H_{16}NS_2O_9K} + H_2O = \underset{\substack{\text{Allyl mus-}\\\text{tard oil}}}{C_3H_5—NCS} + KHSO_4 + C_6H_{12}O_6$$

Tristearin, $C_{57}H_{110}O_6$ Percentage of oxygen is 10.79.
Glucose, $C_6H_{12}O_6$ Percentage of oxygen is 53.33.

188. Transformation of sugar into fat. Since fat is formed in organisms from carbohydrate it may be presumed that glucose or glucose-yielding material furnishes the starting point for such a production. It is possible also that glucose fragments are utilized in the process. In plants the starting point may be formaldehyde synthesized from carbon dioxid and water (182).

If we assume, for purposes of illustration, that glucose can be converted into any or all of the common fats, it is desirable to ascertain the simplest terms in which such a transformation of glucose may be expressed.

Assuming, further, that all of the carbon of the glucose is utilized in such a production of fat, it is obvious that at least $9\frac{1}{2}$ molecules of glucose would be necessary for the synthesis of tristearin. The following calculations give the simplest terms in which this process may be conceived to occur:

Formula of tristearin..... $C_{57}H_{110}O_6$
Corresponding number of atoms composing $9\frac{1}{2}$ molecules of glucose ..... $C_{57}H_{114}O_{57}$
Excess of atoms over the immediate synthetic requirements..... H_4O_{51}, or, $2H_2O + 49\,O$

These calculations imply that a formation of fat from glucose must be attended by very decided reduction of the carbohydrate.

The above calculations and similar data for such syntheses of other fats may be expressed empirically as follows:

Tristearin, $9\frac{1}{2}C_6H_{12}O_6 = C_{57}H_{110}O_6 + 2H_2O + 49\,O$
Triolein, $9\frac{1}{2}C_6H_{12}O_6 = C_{57}H_{104}O_6 + 5H_2O + 45\,O$
Tripalmitin, $8\frac{1}{2}C_6H_{12}O_6 = C_{51}H_{98}O_6 + 2H_2O + 43\,O$
Tributyrin, $2\frac{1}{2}C_6H_{12}O_6 = C_{15}H_{26}O_6 + 2H_2O + 7\,O$

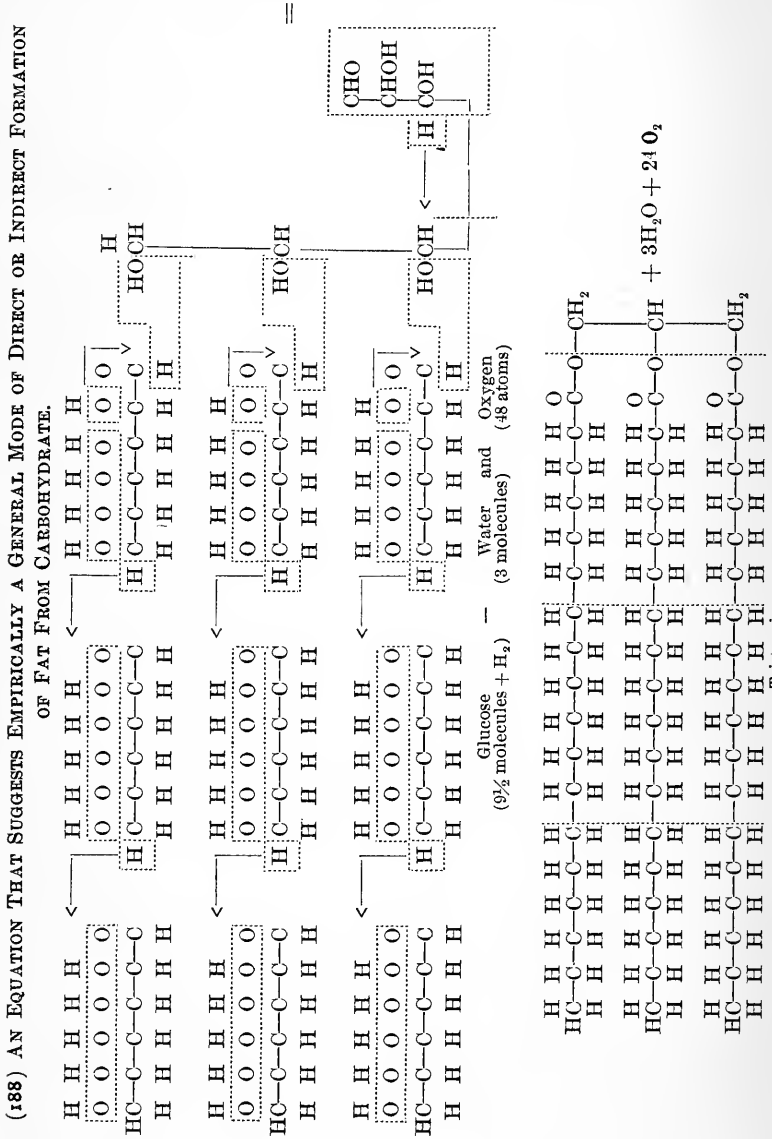
The above facts may also be expressed empirically in a manner illustrated by the following equation:

$$19C_6H_{12}O_6 + 98H_2 = C_{57}H_{110}O_6 + 102H_2O$$

The striking facts in the above quartette of equations are (*a*) the large residues of oxygen, which emphasize the great degree of reduction necessary to convert sugar into fat, and (*b*) the half-molecular

quantity of glucose-carbon necessary to make up the full quota of fat-carbon. The constancy of the half-molecular quantity of glucose-carbon required by the simplest terms of the synthesis, suggests that this carbon residue is utilized for the production of the *glycerol part of the fat molecule*, a deduction that is emphasized by the water mole-

(188) AN EQUATION THAT SUGGESTS EMPIRICALLY A GENERAL MODE OF DIRECT OR INDIRECT FORMATION OF FAT FROM CARBOHYDRATE.



cules of each equation, which seemingly result from a glycerol-fatty acid combination such as occurs in ester formations in general (0 : 154).

These deductions seem to be verified by the graphic arrangements and empirical suggestions on the preceding page.

E. TYPICAL CARBOHYDRATES FOR USE IN THE TESTS.

189. Kinds. In experiments **206–249** use solid samples or 2 per cent. solutions of each of the following typical carbohydrates: *Glucose, sucrose* and *dextrin*. Use also solid *starch* or 2 per cent. starch paste (194). * Practically all the properties of the various important kinds of carbohydrates that are of biochemical interest are illustrated by these four typical products.

Preparation. 190. *Glucose.* The typical monosaccharid occurs, like nearly all the carbohydrates, widely distributed in plants. Nevertheless it cannot be obtained very conveniently in large quantities from plants and is commonly manufactured on a large scale from starch or sucrose by a general process similar to the following: Starch is boiled with comparatively dilute sulfuric acid. In this process starch is hydrated into various simpler carbohydrates, including glucose (170). The thoroughly hydrated mixture is neutralized with calcium carbonate and filtered through animal charcoal to remove calcium sulfate and also colored matters that were developed as by-products in the heating process. The decolorized solution is evaporated to the proper consistency and on cooling crude glucose solidifies as an amorphous mass. The crude product always contains dextrins, which may be removed by treating the mass with hot 90 per cent. alcohol. Dextrins do not dissolve in the alcoholic liquid, glucose does. Glucose may be obtained by evaporation of the alcoholic filtrate.

191. *Sucrose* is usually obtained from the sugar-cane or beet-root. The former contains about 16–18 per cent. of sucrose, the latter about 13–14 per cent. A general process that is employed for the preparation of sucrose is the following: The juice or hot aqueous extracts of the cane or root are obtained. The liquid is boiled with milk of lime, in which process proteins are coagulated (322) and

* Other important carbohydrates, such as glycogen and maltose, will receive due attention later.

lime salts of various acids such as oxalic and phosphoric are precipitated. The filtrate is treated with carbon dioxid to precipitate calcium ;* some decolorization also results. The filtrate from the calcium precipitate is then evaporated to the proper consistency for crystallization of the sugar. Ordinary molasses is the syrupy mother liquor drained from the crystals of crude sucrose obtained in this manner.

Crude sucrose has a brown color. In the refining process the aqueous solution of the crude product is heated with milk of lime or some other decolorizing agent. The clarified liquid is filtered through animal charcoal and the pure sugar crystallized from the colorless filtrate.

192. *Dextrin.* Commercial dextrin, consisting chiefly of a mixture of several dextrins, is usually prepared by a process similar to the one described above for the production of glucose (190). During the heating process samples of the acid mixture (starch and dilute sulfuric acid) are repeatedly treated with iodin (196). As a rule the hydration process is stopped as soon as a sample of the mixture fails to yield a *blue* color with iodin. At this point the dextrin is precipitated with alcohol (196, 239). This precipitate frequently contains a little glucose, and also soluble starch if the hydration process was not carried far enough. Glucose may be removed by repeated reprecipitation of the dextrin (from aqueous solution) with alcohol and the soluble starch can be eliminated by further treatment of the product with hot acid.

Demonstrations. 193. Preparation of oxalic acid (175) from carbohydrate. 194. Preparation of starch from potato and of "starch paste" from the starch thus obtained. 195. Microscopic characters of various forms of starch. 196. Preparation of glucose and dextrins from starch by methods 190 and 192, with observations of the effects of iodin and of alcohol on samples of the mixtures taken at brief intervals. 197. Preparation of glucose and fructose from sucrose and determination of the effect on sucrose of boiling concentrated *hydrochloric acid*.

*Soluble carbohydrates unite with calcium hydroxid and other alkalies to form compounds similar to alcoholates (0 : 81). They are called glucosates, sucrates and so on. Each is decomposed by carbon dioxid. Typical formulas:

$C_6H_{12}O_6$, CaO — Calcium glucosate

$C_{12}H_{22}O_{11}$, 2CaO — Calcium sucrate

198. The polariscope and optical properties of carbohydrates. 199. Tests of the diffusibility of carbohydrates. 200. Tests of the fermentability of carbohydrates, with qualitative and quantitative determinations of products of fermentation. 201. Effects upon carbohydrates of different kinds of bacteria. 202. Properties of cellulose. 203. Preparation of pentosan and pentose. 204. Production of furol from pentoses and pentosans, and application of the anilin test for furol. 205. Furol and its properties.

F. ELEMENTARY COMPOSITION OF CARBOHYDRATES.

206. **Carbohydrates consist wholly of carbon, hydrogen and oxygen.** Apply tests 50 and 51 to a sample of one of the products. Prove the absence of nitrogen, sulfur, phosphorus and iron, tests 53, 62, 63, 64. Compare with 86.

G. PHYSICAL PROPERTIES OF CARBOHYDRATES.

207. Carbohydrates, like fats, are contained in all organisms. Fats are more conspicuous in animals, carbohydrates in plants.

Fats are very much alike in physical and chemical properties. Carbohydrates, on the other hand, differ considerably, both physically and chemically.

208. **Carbohydrates do not impart greasy stains to paper.** (87). *

209. **Pure carbohydrates are neutral compounds** (88).

210. **Microscopic appearance.** Examine under the microscope powdered samples of the carbohydrates (215).

211. **Solubilities** (65).

212. **Carbohydrates do not form emulsions** (93).

213. Examine starch paste under the microscope (195).

Crystallinity. 214. **Demonstration. Methods for crystallizing carbohydrates.**

215. Examine under the microscope a sample of one of the crystallized sugars.

Demonstrations. 216. **Effects of heat on carbohydrates and an examination of the products of their destructive distillation.** 217. **Determination of the melting point of a sugar.**

* Repeat the test indicated and notice agreement or disagreement with former results.

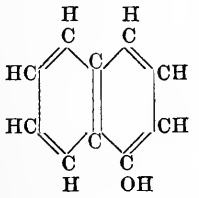
H. CHEMICAL PROPERTIES OF CARBOHYDRATES.

Demonstrations. 218. Microscopic appearance of various solid carbohydrates treated with iodin. 219. The phloroglucin and orcin coloration tests for pentoses and pentose-yielding substances. 220. The resorcin coloration test for fructose and fructose-yielding substances. 221. Precipitation of carbohydrates from alkaline solutions with benzoyl chlorid and methods for the recovery of the carbohydrates from the benzoic acid esters thus produced.

Coloration tests. 222. *Molisch-v. Udránszky test.* To about 2 c.c. of the carbohydrate solution in a test-tube add 2 drops of a 10 per cent. alcoholic solution of α-naphthol. Shake the mixture. Pour carefully and gradually down the side of the tube about 2–4 c.c. of *concentrated* sulfuric acid. At the line of junction of the two liquids a violet or raspberry-red coloration will appear if the test is positive. Shake the mixture. Add more acid.

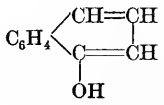
This is a color test for *furol.** All carbohydrates yield furol on treatment with concentrated sulfuric acid. Many proteins yield furol under the same treatment (306). The test is given not only by furol but also by all carbohydrates, and all the furol-yielding substances.

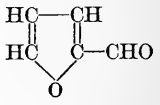
The characteristic colorations are due to compounds of α-naphthol and furol, the exact natures of which have not been ascertained. The chemical characters of furol and α-naphthol are indicated by the following formulas:



α-Naphthol
$C_{10}H_7OH$

or

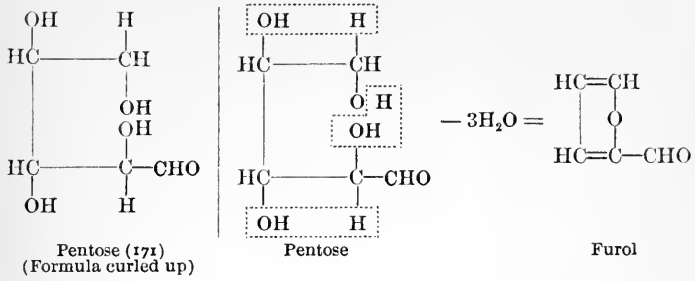




Furol
$C_4H_3O—CHO$

The formation of furol from a typical carbohydrate (pentose), by the action of mineral acids such as sulfuric and hydrochloric (demonstration 204), is indicated by the equation on the opposite page.

*Also called furfurol, furfuraldehyde and furfural.



223. *Iodin test.* A few polysaccharids yield, with iodin, blue or red colored products, which contain iodin in variable proportions and combinations. None of the sugars forms colored products with iodin.

Add to the solids and to the liquids (**189**) ordinary "iodin solution," *i. e.*, iodin dissolved in potassium iodid solution.* Divide into four parts the *colored* solutions containing iodids of polysaccharids and treat the parts as follows :

a. Heat one portion to boiling. Cool. Add an equal volume of fairly concentrated albumin solution. Heat again, then cool.

b. Make a second portion slightly alkaline. Acidify. Render alkaline.

c. Treat a third portion with a small amount of alcohol. Dilute greatly with water.

d. To the remaining portion add solution of silver nitrate in moderate excess.

224. *Moore's test.* Boil with an equal volume of potassium hydroxid solution. A yellow to deep brown coloration may result. If heated long enough a resinous mass may be deposited as in the case of aldehydes (**0 : 106**).

Coloration in Moore's test is due to oxidation of the carbohydrate with formation of alkali salts of glucic acid, $C_{12}H_{18}O_9$ and saccharumic acid, $C_{14}H_{18}O_{11}$ (?), each of which has a brownish color.

This reaction is given only by the carbohydrates that contain free carbonyl groups (**172**). Consequently sucrose and the polysaccharids do not yield it. The carbohydrates that yield the reaction are quickly destroyed by the reagent.

* The reaction given by starch cannot be obtained with a pure aqueous solution of iodin.

Reduction tests. **225.** All the carbohydrates that contain free carbonyl groups exhibit the property of reducing certain compounds of heavy metals, especially when the latter are dissolved in alkaline liquids (172).

The reduction tests are seriously impaired by various substances, among the most common of which are acids, ammonium salts and proteins (288).

Acid solutions that are to be tested must always be made neutral or slightly alkaline (preferably with an alkali hydroxid, never with ammonium hydroxid), *before* adding the *alkaline* metallic solution, because acids themselves exert reducing action on the compounds of the heavy metals employed in the tests and also diminish, in some cases may completely neutralize, the required alkalinity of the solution of the metallic compound.

Ammonium compounds are acted upon by the hydroxid in the solution of the heavy metal, with consequent production of free ammonia. Before using the alkaline solution of the metallic compound sufficient alkali hydroxid must be added to the solution under examination to effect complete decomposition of any ammonium compounds present in it and to insure the presence of a moderate excess of the reagent.

Proteins tend to hold the metallic reduction products in solution (298), or yield precipitates in the alkaline liquids that resemble the reduction products (233). As a rule it is necessary to make certain that proteins are absent (297) before applying the reduction tests described below.

226. *Silver mirror test.* Clean a test-tube thoroughly by boiling in it concentrated nitric acid, afterward potassium hydroxid.

To about 5 c.c. of silver nitrate solution in the perfectly clean tube add a few drops of ammonium hydroxid, *i. e.*, just sufficient to dissolve the precipitated silver oxid. Equations:

$$2AgNO_3 + 2NH_4OH = \underset{\text{Silver oxid}}{Ag_2O} + 2NH_4NO_3 + H_2O$$

$$Ag_2O + 4NH_4OH = \underset{\text{Silver-ammonium hydroxid}}{2Ag(NH_3)_2OH} + 3H_2O$$

To the alkaline solution of silver-ammonium hydroxid thus prepared add a few drops of the carbohydrate solution. Heat the tube and contents in *boiling water*. If reduction occurs it is shown at

once or after a few minutes by deposition of metallic silver, usually in the form of a mirror, if the tube was perfectly clean at the beginning of the experiment. Typical equation (**L : 225**) : *

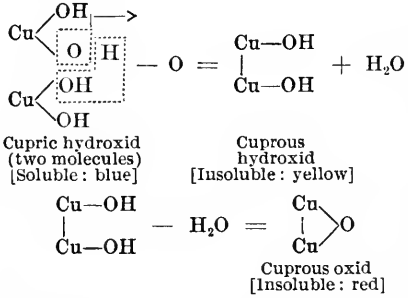
$$2Ag(NH_3)_2OH + 3H_2O + R{-}CHO = 2Ag + R{-}COONH_4 + 3NH_4OH + H_2O$$

227. *Trommer's test.* To about 3 c.c. of potassium hydroxid solution (10 per cent.) add several drops of a solution of cupric sulfate (1 per cent.). Equation:

$$CuSO_4 + 2KOH = \underset{\text{Cupric hydroxid}}{Cu(OH)_2} + K_2SO_4$$

To the slightly bluish mixture just prepared add about an equal volume of carbohydrate solution. Sugars manifest solvent action on the cupric precipitate (**159**).

Boil the liquid for a minute or two. If reduction occurs the blue color disappears or is diminished in intensity, and a yellow or, more frequently, a red precipitate is produced. Equations:



It frequently happens that the boiled mixture exhibits other colors, such as green and brown, which are due to mixtures of some of the colors already referred to. On allowing the mixture to stand, the reduction product quickly subsides.

The copper compound is reduced by the carbohydrate. The *carbohydrate is oxidized* by the copper compound, with effects on the carbohydrate similar to those in Moore's test (**224**).

When only very slight proportions of cupric hydroxid and reducing carbohydrate are present together in a mixture, boiling may result in complete discharge of the faint blue color or in entire replacement of it by a yellowish tint, without causing a cuprous

* R is intended to represent the main part of the carbohydrate.

deposit. In such cases the cuprous compound is held in solution. The yellowish tint results from the action of the alkali hydroxid on the carbohydrate, as in Moore's test (224).

Precautions. Care must be taken to prevent the presence of excess of cupric hydroxid. The deeper the blue color of the mixture the greater the difficulty of detecting a cuprous precipitate, particularly when only a slight proportion of reducing carbohydrate is present and the amount of resultant precipitate is relatively slight. Excess of copper may be deposited, on boiling, as a *black* precipitate of cupric oxid, which would also prevent detection of a cuprous precipitate.

228. Test the latter statement as follows: Make, by the method previously referred to (227), a mixture of alkali hydroxid containing cupric hydroxid. Boil the mixture and notice the conversion of the blue hydroxid to the black oxid. Equation:

$$Cu\begin{matrix}OH\\OH\end{matrix} - H_2O = CuO$$

Cupric hydroxid — Cupric oxid (Insoluble: black)

These difficulties may not be ignored and are overcome in Fehling's test (230), which can be better understood and appreciated after experience with Trommer's test.

229. Repeat test 227 with a solution of sodium potassium tartrate (Rochelle salt) instead of sugar. Notice that boiling has no effect on the deep blue tartrate solution. Compare these effects with those of the preceding experiment and also with those already noticed in a similar experiment with glycerol (159).

230. *Fehling's test* (L: 226). Mix 1 c.c. each of the "copper" and "alkaline" portions of Fehling's solution.* Boil for about a

* The "copper" and "alkaline" portions of Fehling's solution, as prepared for use, were made as follows:

"*Copper*" *portion.* 34.64 grams of pure crystalline copper sulfate were dissolved in 500 c.c. of distilled water. "*Alkaline*" *portion.* 175 grams of pure crystalline sodium potassium tartrate (Rochelle salt) and 50 grams of pure sodium hydroxid were dissolved in 500 c.c. of distilled water.

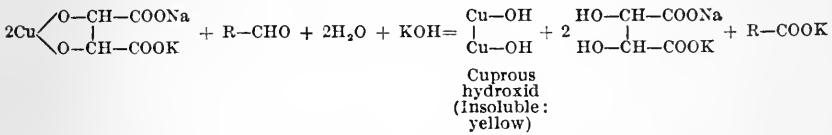
Fehling's solution is a mixture of *equal volumes* of the "copper" and "alkaline" solutions. The reactions involved in its preparation are indicated by the following equations:

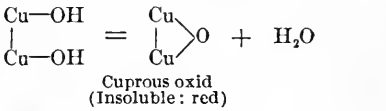
$$CuSO_4 + 2KOH = Cu(OH)_2 + K_2SO_4$$

Cupric hydroxid (Bluish-white: insoluble)

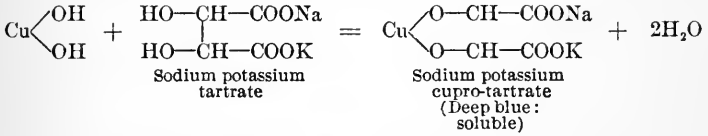
minute. No change in the color of the solution can be seen.* Add an equal volume of liquid containing carbohydrate. Boil again for a minute or two. A greenish, yellowish, brownish or bright red coloration results according to the degree of reduction, the proportion of unchanged copper compound, etc., as was previously explained (227). Let the mixture stand and examine the deposit.

The chief chemical facts involved in the use of Fehling's solution are indicated below (see footnote, pages 68–69):





231. *Nylander's modification of the Böttger-Almén test.* To 10 parts of the liquid containing the carbohydrate add 1 part of Nylander's solution † and boil continuously for from 2 to 5 minutes.



Prepared in the proportions indicated 5 milligrams of glucose will reduce all the copper compound in exactly 1 c.c. of Fehling's solution under certain fixed conditions. The solution is very frequently used for quantitative determinations (235). For *qualitative* purposes the solution may be diluted with water or preferably with hydroxid solution before using.

Fehling's solution *readily decomposes* soon after its preparation, in which condition it undergoes *spontaneous* reduction on boiling and cannot be relied upon for the detection of reducing substances. By keeping the "copper" and "alkaline" portions in separate bottles, however, this deterioration is prevented. A trifling quantity of phenol or other "indifferent" preservative may be added to the "alkaline" portion to prevent development of fungi and any decomposition of the alkaline solution that might possibly occur through their influence.

* If a change occurs the solution is unfit for use.

† Nylander's solution is usually made in the following proportions: 4 grams of sodium potassium tartrate (Rochelle salt) are dissolved in 100 c.c. of 5 per cent. sodium hydroxid solution. To this solution 2 grams of bismuth subnitrate are added and the mixture is heated on a water bath until it is saturated with the bismuth compound. The filtrate is the reagent.

If reduction occurs the solution turns yellow at first, then brown, and finally black. On standing, a black precipitate of metallic bismuth, or of a mixture of bismuth and black oxid of bismuth is thrown down.

The reactions involved in the use of Nylander's solution are not definitely known, but no doubt are closely analogous to those indicated on page 69 for Fehling's solution.

232. *Barfoed's test.* To about 5 c.c. of the solution add a few drops of Barfoed's reagent.* Heat the mixture in a water bath for about 30 minutes. Glucose is the only carbohydrate that will reduce Barfoed's reagent. The test is not so delicate as the other reduction tests. The reagent soon deteriorates and cannot be relied upon unless it has been freshly prepared.

Demonstrations. 233. Substances and conditions that interfere with the reduction tests. 234. Use of Haines' and Pavy's solutions. 235. Quantitative determination of sugar with Fehling's solution.

236. Percentage amounts of carbohydrates obtained from some mammalian parts and from various vegetable food-stuffs.†

Fats occur in relatively greater proportions in animals than in plants.‡ The reverse is true of carbohydrates.

The figures in the table on the opposite page represent general average percentage amounts of carbohydrate matters contained in the materials named.

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* Barfoed's reagent is commonly prepared in the following proportions: 20 grams of cupric acetate are dissolved in 300 c.c. of water. To this solution is added 10 c.c. of 35 per cent. acetic acid. This reagent is particularly unlike the other copper reagents (Trommer's, Fehling's, Haines', Pavy's) in being *acid* in reaction.

† The proportions of carbohydrate in some of the products named in the table on the opposite page vary considerably under ordinary conditions.

‡ The largest proportions of fats in plants occur in the so-called oil-seeds. In such seeds as walnut and cocoanut the proportion of fat in the true seed-meat (endosperm) amounts to from 30 to 40 per cent. of the fresh material. In other vegetable parts the proportional content of fat is much less, *e. g.*, in wood, which contains only traces (117).

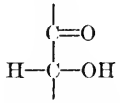
Animal parts * (carbohydrate is *chiefly* glycogen, lactose or glucose):

Blood	0.1–0.15	(Glucose)
Leucocytes (thymus)	0.8	(Glycogen)
Muscle	1.0–3.0	(Glycogen)
Liver	1.5–4.5	(Glycogen)
Milk	3.5–5.0	(Lactose)
Urine:		
Normal	trace	
Abnormal (diabetic)	10 (or more)	(Glucose) †

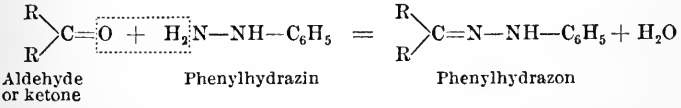
Vegetable parts (carbohydrate is *chiefly* starch in each case):

Cucumber	2
Cabbage	5
Apple	13 ‡
Potato	20
Oat (grains)	56
Barley (grains)	65
Rye (grains)	69
Rice (grains)	77

237. **Phenylhydrazin test.** This is one of the most important of the carbohydrate tests. It has already been stated (page 50) that the monosaccharids contain the group,



We have also learned that phenylhydrazin reacts upon aldehydes and ketones, uniting directly with the carbonyl groups as follows (**0 : 102**):



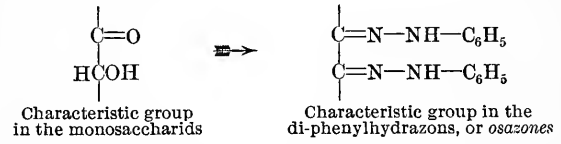
Such combinations may be made to occur between phenylhydrazin and the carbohydrates that contain free carbonyl groups. But in the case of a carbohydrate that reacts with phenylhydrazin, the group, HCOH, which is attached to the carbonyl radical, is also

* Carbohydrate occurs universally in organisms. In nearly all *animal* parts, however, the proportions that can be detected are *extremely slight*.

† A daily elimination by diabetic patients of 2 pounds or more of glucose has been observed frequently.

‡ In *sweet* apples most of the starch of the green fruit has been converted into sucrose by the ripening process.

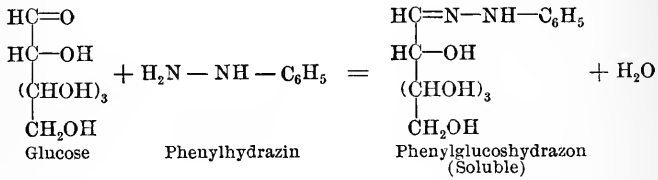
involved. A second molecule of phenylhydrazin withdraws from that group the two hydrogen atoms, thus converting the alcohol radical into a carbonyl group, while the phenylhydrazin itself is converted into anilin and ammonia. A third molecule of phenylhydrazin then reacts with the new carbonyl group as in the first case. The changes in the natures of the characteristic groups may be indicated as follows:



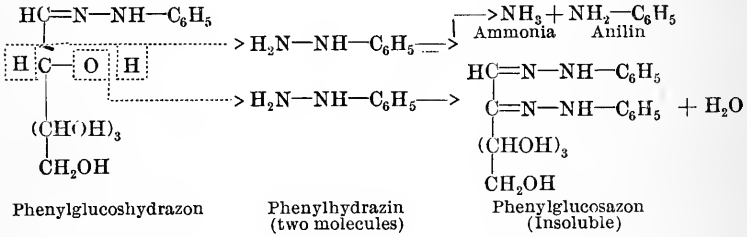
Characteristic group in the monosaccharids

Characteristic group in the di-phenylhydrazons, or *osazones*

The reactions are indicated by the following typical equations:



Glucose Phenylhydrazin Phenylglucoshydrazon (Soluble)



Phenylglucoshydrazon Phenylhydrazin (two molecules) Phenylglucosazon (Insoluble)

The common osazones are yellow substances that may be crystallized readily. Unlike the sugars, they are only slightly soluble in water and biological liquids, and may be purified without difficulty by recrystallization from various solvents, such as pyridin. Each of the purified products has a characteristic crystalline appearance, melts at a particular temperature, and has specific optical properties. As a rule the identity of the antecedent sugar may be readily ascertained by determinations of the melting point of the purified osazon.

The insolubility of the osazones makes it an easy matter to precipitate many of the carbohydrates from mixed solutions containing

other substances. The corresponding sugars may be obtained from the osazones by various methods.*

238. Apply the phenylhydrazin test as follows: A. Dissolve in a very small volume of water a mass of phenylhydrazin hydrochlorid † equal to that of a pea. Add to the solution about the same bulk of sodium acetate. Filter, if the solution does not get clear after repeated agitation.

B. Pour solution A into about 5 c.c. of the carbohydrate liquid. Immerse the tube in boiling water and keep it there for about

* "It is a somewhat remarkable fact that methyl-phenylhydrazin,

$$NH_2—N(CH_3)—C_6H_5,$$

yields osazones only with ketoses, and not with aldoses. The latter form colorless hydrazones with this compound, and these can easily be separated from the intensely yellow osazones. Methyl-phenylhydrazin therefore affords a valuable means of detecting ketoses.

"When the osazones are carefully warmed with hydrochloric acid, two molecules of phenylhydrazin are split off, with formation of compounds, *osones*, containing two carbonyl groups. In this way, glucosazone yields *glucosone*,

$$CH_2OH—(CHOH)_3—CO—HCO$$

The osones can be reduced by treatment with zinc-dust and acetic acid, and experience has shown that addition of hydrogen always takes place at the terminal C-atom. Glucosone yields fructose, $CH_2OH—(CHOH)_4—CO—CH_2OH$. This reaction affords a means of converting aldoses into ketoses:

Aldose ⇒ Osazone ⇒ Osone ⇒ Ketose

Inversely, an aldose can be obtained from a ketose. On reduction, the latter yields a hexahydric alcohol, which is converted by oxidation into a monobasic hexonic acid. This substance splits off water, yielding the corresponding lactone, which on reduction gives the aldose." [Holleman.]

Ketose ⇒ Hexahydric alcohol ⇒ Hexonic acid ⇒ Lactone ⇒ Aldose

Monosaccharids may be transformed directly into one another through the action of very dilute alkalies. Such transformations also occur in the body.

† Phenylhydrazin is a liquid base. It is insoluble in water and ordinary biological liquids, but dissolves readily in dilute acids, such as acetic and hydrochloric. It forms soluble salts with the acids. The base itself cannot be used satisfactorily in the tests in the absence of acetic acid. As a rule the tests are carried out with the solid (and soluble) hydrochlorid, $C_6H_5—NH—NH_2$, HCl. The latter substance will not react with carbohydrates in the absence of acetic acid or acetate. In the tests sodium acetate is added in excess. It reacts with HCl of the hydrochlorid, thus producing the required acetic acid and also sodium chlorid as a by-product. A moderate excess of the reagent is essential to the success of the test. Failure may be due in some cases to formation of soluble hydrazones because of lack of sufficient hydrazin.

an hour. Finally set aside the tube in hot water in a beaker and let the contents of the tube *cool slowly*. Examine under the microscope any deposit that may be formed.

I. PRECIPITATION FROM AQUEOUS SOLUTIONS.

239. The carbohydrates may be readily precipitated by various reagents from their aqueous solutions. Among the most valuable precipitants are alcohol (314) and ammonium sulfate (317).

240. **Alcohol.** Transfer to small beakers 10 c.c. of comparatively concentrated solutions of each of the carbohydrates under examination. Add to each liquid 10 c.c. of 95 per cent. alcohol. Stir thoroughly. Filter, if necessary, on *dry* apparatus. Test the solubility in water and coloration with iodin of any precipitate that may be produced.

241. Repeat the additions of alcohol, in 10 c.c. portions at a time to each beaker (240), until a total of 50 c.c. of alcohol has been employed in this way. Stir thoroughly after each addition of alcohol. Filter on *dry* apparatus whenever precipitation is induced, and test the solubility in water and coloration with iodin, of any precipitate that may be produced.

The more complex the carbohydrate the more readily it may be precipitated by alcohol. Relatively large proportions of alcohol are required to precipitate monosaccharids. All polysaccharids are *easily* precipitated completely from their aqueous solutions by alcohol.

242. Remove the alcohol from each of the final filtrates or unprecipitated liquids (241), by evaporation on a water bath. Test the concentrated liquids with iodin and Fehling's solution.

243. **Ammonium sulfate.** Transfer to small beakers 12 c.c. of comparatively concentrated solutions of each of the carbohydrates under examination. Follow the plan of the experiments with alcohol, but use, instead of alcohol, ammonium sulfate as follows:

244. *A. Half-saturation with ammonium sulfate.* Treat 10 c.c. of the aqueous carbohydrate solution with 10 c.c. of *saturated* aqueous solution of ammonium sulfate. Stir thoroughly. Let the mixtures stand about 10 minutes. Filter if necessary, on *dry* apparatus. Determine the solubility in water of any precipitate that may be formed; also the iodin reaction of the product.

245. *B. Saturation with ammonium sulfate.* Treat with iodin or Fehling's solution a small portion of each of the unprecipitated liquids, or any filtrates that may have been obtained (244). To the main bulk of the carbohydrate solution add powdered ammonium sulfate until the liquid is *saturated* at room temperature. Avoid excess of sulfate. Filter. Apply to the filtrates the tests indicated in section 244.

Common starches are of two kinds: (*a*) ordinary starch, which is insoluble in cold water and of which practically all the undissolved matter in starch paste is composed; (*b*) "soluble" starch or "amidulin," which may be formed from insoluble starch by initial *hydration,* is produced in this way in starch paste, and is contained in the filtrate from starch paste. Each of the starches yields a blue compound with iodin, that with insoluble starch failing to dissolve in water, whereas the product with soluble starch dissolves readily in water. The viscid masses of insoluble starch in starch paste are immediately dehydrated and made flocculent by half-saturation of the paste with ammonium sulfate. Soluble starch is completely precipitated in 24 hours, only incompletely in a few minutes, by half saturation of its solution with ammonium sulfate.

Dextrins are of two general types: (*a*) erythrodextrins (I–III), which yield soluble reddish compounds with iodin, and (*b*) achroödextrins, which fail to yield colored compounds with iodin. Of the erythrodextrins, I and II are completely precipitated by saturation of their solutions with ammonium sulfate; erythrodextrin III and achroödextrins are not precipitated under such conditions.

The sugars are not precipitated from aqueous solutions by ammonium sulfate.

The iodin colorations obtained with the erythrodextrins are the following: Erythrodextrin I, purple; erythrodextrin II, red; erythrodextrin III, reddish brown. Erythrodextrin I is completely precipitated from aqueous solution by saturation with magnesium sulfate; erythrodextrin II is not precipitated under such conditions.

Demonstrations. 246. Various additional methods of precipitating carbohydrates. 247. Detection of carbohydrate in the presence of fat, fatty acid, soap and glycerol.

J. HYDRATION OF CARBOHYDRATES (170).

248. Monosaccharids may be obtained by hydration from all other classes of carbohydrates and from carbohydrate-yielding substances, such as glucosids (185) and various proteins (250).

249. Place in small beakers 20 c.c. of each of the carbohydrate solutions. Add to each solution an equal volume of 0.2 per cent. hydrochloric acid. Cover the beakers with watch glasses. *Boil* each solution gently about 30 minutes. Finally neutralize the solutions and apply to each tests 223, 230 and 232.

K. RELATIONSHIP BETWEEN CARBOHYDRATE AND PROTEIN.

250. Most of the proteins (252) respond positively to Molisch's test (222). Some proteins may be made to yield more or less carbohydrate material on chemical decomposition. In plants carbohydrates are combined with various amino substances (264) to form proteins. Among the transformation products of proteins in organisms are carbohydrates. Some of the proteins may be regarded as complex glucosids (184). A few of the proteins contain carbohydrate radicals in so large proportions and yield so much carbohydrate material on decomposition that, as a group, they are called *gluco*-proteins (255). The characters of the various carbohydrate radicals that are certainly present in many proteins have not been satisfactorily determined, but among the known hydration products are glucosamin (177) and galactosamin. The latter is a stereoisomer of the former (171).

These facts lead to the deduction that carbohydrate is utilized directly or indirectly in organisms for the synthesis of some perhaps of all kinds of proteins.

CHAPTER IV.

PROTEINS.*

A. Demonstration of Protein Products.

251. Representatives of the groups of proteins named in the summary on pages 79–80.

B. General Characteristics of Proteins.

252. Distribution. "Few substances are so widely distributed in nature as proteins and certainly none are of more consequence from a biological point of view. The tissues of all plants and animals contain these substances in large proportions and of the invariable organic constituents of every living functionally active cell the albuminous (protein) are undoubtedly the most important.

253. Molecular complexity. "That the proteins are among the most complex compounds with which the chemist has to deal, and therefore, also, the most elusive in chemical research, are deductions to which the experiences of all protein investigators seem to point conclusively. In spite of the fact, however, that the proteins have long been the subjects of persistent and carefully conducted chemical investigation, our knowledge of the molecular configuration of the proteins still remains decidedly indefinite and all attempts thus far completely to unravel the constitution of the protein molecule have resulted negatively. Each of the various theories which have been proposed in regard to structural formulas depends chiefly upon the nature of the products obtained by protein decompositions, since all of the numerous attempts to prepare *true* albuminous material artificially have invariably resulted in failure. Since the decomposition products of protein matter are multitudinous and, under different conditions so various, it is not at all difficult to

*The words "protein" and "proteid" are synonymous in English, although the latter term is gradually becoming obsolete. The phrase "albuminous substance" is also frequently used synonymously in English. In German "Proteid" and the equivalent of "albuminous substance" ("Eiweisskörper") are used to designate sub-groups of "proteins" ("Proteïne").

comprehend why the biological chemist is so much in the dark as to the real configuration of the protein molecule and why, in the absence of sufficient data afforded by artificial synthesis, he is able to form only hypotheses as to the manner in which the analytic nuclei obtained from proteins are held in the undecomposed substances.

254. Synthesis in organisms. "Although true protein matter has never been prepared in the laboratory from any of its decomposition products, its synthesis is constantly taking place in plants, and to a certain extent, in animals as well. The ultimate origin of proteins may be traced to the vegetable kingdom, however, for plants are constantly transforming *inorganic* matter into albuminous substances, as a part of the process of their development, whereas in animal metabolism, albuminous syntheses are wholly dependent upon protein derivatives that are assimilated after digestion of protein food." *

C. CLASSIFICATION OF PROTEINS.

255. Groups. The fats and carbohydrates may be satisfactorily classified on the basis of intramolecular differences. Such a chemical classification cannot, however, be made of proteins at present, because of the meagerness of our knowledge of the intramolecular nature of these very complex substances. The empirical classification of proteins that is suggested on pages 79 and 80 depends, in the main, upon superficial distinctions, such as similarity or dissimilarity in solubility, precipitability, derivability from more complex proteins, convertibility into simpler proteins and so on. The chief merits of the classification proposed are its convenience, and the general physical, chemical and biological relationships that it makes evident.†

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* Gies: Yale Scientific Monthly, 1898, iv, pp. 204–205; also, Gies and collaborators, Biochemical Researches, 1903, i, pp. 719–720 (Reprint No. 39).

Although the remarks that are quoted above (252–254) were written by the author nearly ten years ago, they require very little qualification now, in spite of the fact that many admirable investigations have been carried out since then in order if possible to throw more light on the molecular nature of protein matter. This fact emphasizes the difficulties of the problems indicated.

† The difficulties involved in perfecting a classification of the proteins are so great that each investigator of the protein substances is apt to have a classification of his own. For this reason the student who uses this volume is advised to consult other authors on this subject.

CLASSIFICATION OF PROTEINS (255).*

I. Primary proteins or true albuminous substances.

A. Proteins that occur in organisms as free compounds, as simple salts of the common inorganic ions (or molecules), or as simple organic compounds, and which cannot as a rule be obtained unchanged from other proteins by laboratory methods.

a. **Albumins** — *Cell albumins, serum albumins.*

b. **Globulins** — *Cell globulins, myosin, fibrinogen, edestin.*

c. **Phosphoglobulins** — *Caseinogen, mucous protein.*

B. Proteins that occur naturally only in combination with other substances (in compound secondary proteins) but which may be obtained from the latter by appropriate laboratory methods.

a. **Histons** — *Globin, leucocyte histon, spermatozoan histon.*

b. **Protamins** — *Clupein, salmin, scombrin, sturin.*

II. Secondary proteins or derivatives of true albuminous substances.

A. Compound derivatives.

a. Natural compound proteins: derivatives or compounds of true albuminous substances that cannot be exactly duplicated by known laboratory methods and which occur in organisms free or as salts of common inorganic ions or molecules.

1. **Nucleoproteins** — *Nucleohiston, cytoglobin.*
2. **Chromoproteins** — *Hemoglobin, hemocyanin.*
3. **Glucoproteins:**
   Non-phosphorized — *Osseomucoid, salivary mucin.*
   Phosphorized — *Ichthulin, helicoprotein.*
4. **Lecithalbumins.**
5. **Iodoproteins** — *Thyreoglobulin.*

b. Artificial compound proteins: additive products which result from combinations of various kinds of proteins with other proteins (or non-protein organic substances) that may be brought about by laboratory methods.

1. **Similar to natural compound proteins.** — Albumin combined with glucoproteins, lecithin and various other colloidal substances that normally occur in organisms.
3. **Special organic salts.** — Albumin combined with coloring matters, picric acid and other organic reagents.

* Only common members of each group are named.

B. Simple derivatives.

a. Simple derivatives of true albuminous substances, that *cannot be made by laboratory methods* and which occur chiefly in the tissues as structural materials; they appear to be condensation products and are often called "**albuminoids.**" Chief among them are, (1) *collagen*, (2) *elastin*, (3) *albumoid*, (4) *keratin*.

b. Simple proteins that are made in organisms from other proteins and *may also be made from other proteins by laboratory methods.*

1. From proteins in general.
   - i. **Proteoses** — *Albumose, caseose.*
   - ii. **Peptones** — *Globulin pepton, myosin pepton.*
2. From most primary and compound proteins, and from groups 4 (*II, B, b*) and *C* (*II*) of simple derivatives. **Proteinates** — *Acidalbumin, alkali albuminate.*
3. From collagen only — *Gelatin.*
4. Solidified by enzymes.
   - i. *Casein* (from caseinogen).
   - ii. *Fibrin* (from fibrinogen).
   - iii. *Plasteins* (from proteoses).
   - iv. *Myosin fibrin* (from myosin).

C. Proteins that do not occur normally in organisms, but which may be made in the laboratory, from various primary and secondary proteins.

a. **Coagulated proteins** — Produced by heat, alcohol or other means, such as *coagulated albumin.*

b. **Special inorganic salts of proteins** — *Copper albuminate, globulin phosphotungstate.*

III. Digestive, also artificial synthetic products, that resemble in some respects a few of the simplest proteins — Peptids.

256. Group properties. The properties characteristic of the subgroups of proteins that are indicated on pages 79 and 80 are so varied and the peculiarities of the individual members of each group are so marked that we shall find it convenient to delay further consideration of the differences among the proteins until we have become more familiar with their general properties as exhibited by the various typical proteins selected for the tests (**269**). In our study of the tissues and body fluids each of the important proteins will be considered from this standpoint in connection with its situation and functions.

D. ELEMENTARY COMPOSITION.

257. Qualitative elementary composition. Like fats and carbohydrates, all protein molecules contain carbon, hydrogen and oxygen. But all protein molecules are unlike those of fats and carbohydrates in containing *nitrogen*, besides. Most protein molecules also contain *sulfur;* many contain phosphorus in addition and some likewise contain *iron, copper, iodin* or other unusual elements in strict "organic combination." Protein *salts*, many of which occur naturally, contain various elements in addition to those just mentioned.

258. Typical formulas. The following empirical formulas of three typical proteins show at a glance the diversity and complexity so characteristic of the proteins as a group:

Pepton ..... $C_{21}H_{34}N_6O_9$
Serum albumin ..... $C_{450}H_{720}H_{116}O_{140}S_6$
Hemoglobin ..... $C_{758}H_{1203}N_{195}O_{218}S_2Fe$

259. Quantitative elementary composition. The figures for percentage elementary composition of some of the more important proteins, arranged in the order of classification (pages 79 and 80), are summarized below:

	C	H	N	O	S	P	Fe
Serum albumin	53.08	7.10	15.93	21.99	1.90	.....	.....
Egg albumin	52.75	7.10	15.51	22.90	1.62	.....	.....
Serum globulin	52.71	7.01	15.85	23.32	1.11	.....	.....
Edestin	51.27	6.85	18.76	22.22	0.91	.....	.....
Fibrinogen	52.93	6.90	16.66	22.26	1.25	.....	.....
Leucocyte histon (Thymus)	52.37	7.70	18.35	20.96	0.62	.....	.....
Globin	54.97	7.20	16.89	20.52	0.42	.....	.....
Clupein	47.24	8.14	25.72	18.90	.....	.....	.....
Pancreatic Nucleoprotein	51.35	6.81	17.82	20.93	1.29	1.67	0.13
Thymus Nucleohiston	48.80	7.03	18.37	21.59	0.51	3.70	.....
Hemoglobin	54.57	7.22	16.38	20.43	0.57	.....	0.34
Hemocyanin*	53.66	7.33	16.09	21.67	0.86	.....	.....
Osseomucoid	47.07	6.69	11.98	31.85	2.41	.....	.....
Ichthulin	53.52	7.71	15.64	22.19	0.41	0.43	0.10
Thyreoglobulin†	51.85	6.88	15.49	23.57	1.87	.....	.....
Elastin	54.14	7.33	16.87	21.52	0.14	.....	.....
Chondroalbumoid	50.46	7.05	14.95	25.68	1.86	.....	.....
Fibrinoses‡:							
Primary... Proto	55.12	6.61	17.98	19.07	1.22	.....	.....
Primary... Hetero	55.64	6.80	17.66	18.69	1.21	.....	.....
Secondary *A* (Thio)	48.96	6.90	16.02	25.15	2.97	.....	.....
Secondary *B* (Gluco)	48.72	7.03	13.76	30.49		.....	.....
Secondary *C*	34.52	5.85	17.24	42.89		.....	.....
Antipepton	46.20	6.74	16.26	30.80	.....	.....	.....
Gelatin	50.11	6.56	17.81	25.24	0.26	.....	.....
Casein	53.07	7.13	15.64	22.60	0.76	0.80	.....
Fibrin	52.68	6.83	16.91	22.48	1.10	.....	.....
Coagulated egg albumin	52.33	6.98	15.84	23.04	1.81	.....	.....

* Content of copper = 0.38 per cent. † Content of iodin = 0.34 per cent.
‡ Compare with the figures for fibrin.

E. CLEAVAGE PRODUCTS OF PROTEINS.

260. It is obvious that the figures given on page 81 for elementary composition of proteins tell very little about the molecular nature of the products referred to. Thus the figures for percentage elementary composition of egg albumin and serum globulin are practically the same :

	C	H	N	O	S
Egg albumin	52.75	7.10	15.51	22.90	1.62
Serum globulin	52.71	7.01	15.85	23.32	1.11

These proteins are decidedly different in certain respects, as we shall see, yet the figures for composition suggest that they are practically the same. From this standpoint these two proteins are similar to isomers such as the aldohexoses (**171**), which, although exactly the same in elementary composition are very different in certain respects because of their unlikenesses in molecular configuration.

Chemists have long appreciated the fact that a more perfect knowledge of protein matter awaits complete determinations of the internal structure of protein molecules. But many of the difficulties of such determinations have been insuperable.

261. As a rule the molecular construction of a compound is ascertained satisfactorily by two general methods of investigation :

1. The substance under examination is subjected to cleavage (*analysis*) and the characters of the decomposition products are carefully ascertained.

2. The cleavage products are reunited by appropriate methods (*synthesis*) and the original substance is thus regenerated.

When these two processes give complete and perfectly satisfactory results the intramolecular qualities of the substance under examination may be accurately established. Both of these methods of investigation have been applied to proteins, but thus far the list of cleavage (*analytic*) products that may be obtained from proteins is far from complete and no one has succeeded in putting more than a few of the very many cleavage products together again. As has already been indicated (page 81), the imperfect synthetic products that have been made resemble some of the simplest proteins, but as yet they are unlike all of the proteins in very important

respects. This synthetic success, incomplete though it has been, makes it appear to be only a question of time and investigation, however, until natural proteins can be perfectly synthesized from their cleavage products, and until typical proteins can be made in the laboratory almost as readily as typical sugars can now be produced synthetically. These are no reasons for thinking that these desirable results are unattainable.

The known cleavage products of the various proteins are qualitatively much the same. The widest variations from the average qualitative results afforded by any given method of cleavage are exhibited by the compound proteins, which yield cleavage products that represent not only the strictly protein part of their molecules, but also the non-protein portion.

The qualities of the cleavage products of proteins vary considerably also with the characters of the methods employed to effect decomposition. The molecules are so intricate in structure that, speaking figuratively, the fragments which result from breaking the molecule to pieces vary in size, shape and other characters as the force of the blow that is administered to the molecule is increased or decreased, or as the blow falls upon one point or another on the surface of the molecule.

As has already been indicated most of the proteins may be converted by laboratory methods into other proteins, which are usually only of a simple type, however, such as proteinates, proteoses and peptones. Such protein products that result from the modification of more complex proteins need not be considered here.

262. In the following summary a few of the common methods that are used to effect profound decomposition of proteins are mentioned with the names of the more important non-protein cleavage products that are obtainable from typical proteins by the processes indicated.

Ignition. — Water, carbon dioxid, ammonia, hydrogen sulfid, inflammable gases, nitrogenized bases, etc.

Fusion with caustic alkali. — Ammonia, carbon dioxid, methyl mercaptan, indol, skatol, phenol, fatty acids (salts) such as acetic, butyric and valeric, leucin, tyrosin, etc.

Putrefaction. — Fatty acids, phenol and indol derivatives, ptomains, carbon dioxid, hydrogen sulfid, hydrogen, ammonia, methane, methyl mercaptan, etc.

The decomposition products obtained by the three methods just indicated result from very profound disorganization of the carbon nuclei or chains of the protein molecules and on that account do not indicate very much as to the *inner structure* of the molecules from which they have been derived. By these drastic methods, the structural elements are altered beyond recognition.

263. The most significant non-protein cleavage products of proteins are those obtained by hydrolysis with boiling dilute mineral acids or through the agency of certain proteolytic enzymes that may be extracted from organisms. These methods of cleavage are less drastic than the three named above. Consequently the resultant segments of the carbon chains that were originally present in the molecule are longer so to speak, also less altered, and more indicative of the way in which the chains are joined together in the molecule. These relatively mild methods of cleavage also seem to exert less influence on the bonds between the carbon and nitrogen atoms, and the cleavage products that are obtained with the aid of these methods are more like simple, unmodified fragments than those produced by the action of the more vigorous means of effecting decomposition.

264. Hydrolysis. The most important cleavage products that result from hydration of practically all true proteins through the action of boiling dilute mineral acids may be classified as follows:

ALIPHATIC PRODUCTS.

Nitrogenous, free from sulfur.

Mono-basic, mon-amino acids.

Amino-acetic (glycocoll), $CH_2(NH_2)COOH$

α-Amino-propionic (alanin), $CH_3-CH(NH_2)COOH$

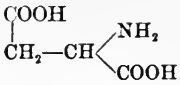
α-Amino-β-oxy-propionic (serin), $CH_2(OH)-CH(NH_2)COOH$

α-Amino-*n*-valeric, $CH_3-CH_2-CH_2-CH(NH_2)COOH$

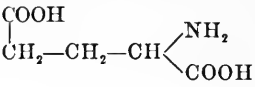
α-Amino-iso-butyl-acetic (leucin), $(CH_3)_2CH-CH_2-CH(NH_2)COOH$

Di-basic, mon-amino acids.

Amino-succinic (aspartic acid),

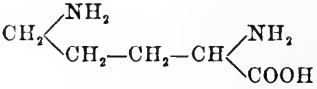


α-Amino-glutaric (glutamic acid),



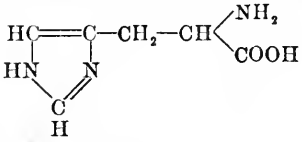
Mono-basic di-amino acid.

α, ∂-Di-amino-*n*-valeric (ornithin),

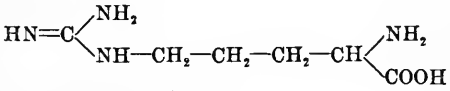


Hexon bases.

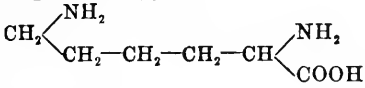
α-amino-β-imidazol propionic acid (histidin),



Guanidin-α-amino-*n*-valeric acid (arginin),



α, ε-Di-amino-*n*-caproic acid (lysin),

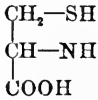


Carbohydrate derivative.

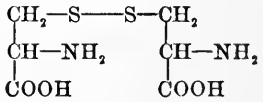
Glucosamin (178).

Nitrogenous, containing sulfur.

α-Amino-β-thio-lactic acid (cystein),

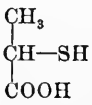


α-Di-amino-β-di-thio-di-lactylic acid (cystin),



Sulfurous, free from nitrogen.

α-Thio-lactic acid,



Ethyl sulfid,

Mercaptans, *e. g.*, methyl mercaptan, $CH_3—SH$

CARBOCYCLIC PRODUCTS.

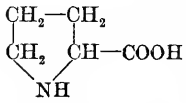
Phenyl-α-amino-propionic acid (phenyl-alanin), $CH_2—C_6H_5$, $CH—NH_2$, $COOH$

p-Oxy-phenyl-α-amino-propionic acid (tyrosin), $CH_2—C_6H_4—OH$, $CH—NH_2$, $COOH$

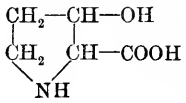
HETEROCYCLIC PRODUCTS (see hexon bases).

Pyrrol derivatives.

α-Pyrrolidin-carboxylic acid (prolin),



Oxy-pyrrolidin-α-carboxylic acid,



Indol derivative.

Indol-β-amino-propionic acid (tryptophan), C_6H_4, $CH_2—NH_2$, $C—CH$, $COOH$, CH, NH

265. Relations of cleavage products to molecular structure.—So far as our present knowledge extends we may say that the substances named on pages 84–86 are the most significant cleavage products of proteins. As a rule, over 50 per cent. of the nitrogen of proteins may be obtained by hydrolysis in the form of mon-amino acids. When proteins are treated with nitrous acid only, relatively slight proportions of nitrogen are evolved. If *amino* groups were contained in proteins to the extent that the large yield of mon-amino acids might be assumed to indicate, a correspondingly large proportion of nitrogen would be evolved as a result of the aforesaid treatment with nitrous acid (**0 : 184**).

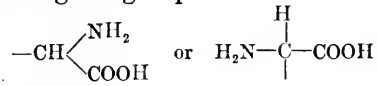
It must be distinctly understood that none of the above-named products exists as such in the protein molecule, but rather that the molecular nucleus of each particular cleavage product is probably combined with other such nuclei to form the whole protein molecular frame-work. It is quite probable also that the products obtained by hydration are merely fragments of larger intra-protein nuclei and that the particular products of hydrolytic cleavage vary in nature

with differences in the characters of attached groups in the various proteins. It is generally believed that the precursors in the protein molecule of the amino groups in the amino-acid cleavage products are mainly *imino* groups (**0 : 181**) and that the latter are converted into amino radicals by the hydration process.

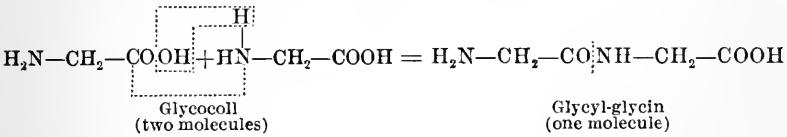
Most of the proteins yield on hydrolysis practically all of the cleavage products already noted. Other proteins have failed to yield some of the products. No two kinds of proteins yield the same proportions of any of the cleavage products.

F. CONSTITUTION OF PROTEINS AND ATTEMPTS AT SYNTHESIS.

266. Peptids. All of the amino-acid cleavage products of proteins (pages 84–86) are α-amino-acids (**0 : 184**) and each is characterized by containing the group



The α-amino-acids may readily be made to unite with one another, in which process the amino group of one molecule unites with the carboxyl group of the other, with elimination of water, as is indicated by the following typical equation :



Glycyl-glycin is both an amid (**0 : 184**) and an amino-acid, and is a member of the group of substances called di-peptids. Similar amid-amino-acids have been obtained, among which the substances with the following formulas are now well known :

Di-peptids :

Alanyl-alanin, $H_2N-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CO\!:\!NH-\underset{\displaystyle CH_3}{\underset{|}{CH}}-COOH$

Leucyl-leucin, $H_2N-\underset{\displaystyle C_4H_9}{\underset{|}{CH}}-CO\!:\!NH-\underset{\displaystyle C_4H_9}{\underset{|}{CH}}-COOH$

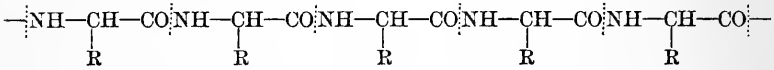
Tri-peptid :

Di-glycyl-glycin, $H_2N-CH_2-CO\!:\!NH-CH_2-CO\!:\!NH-CH_2-COOH$

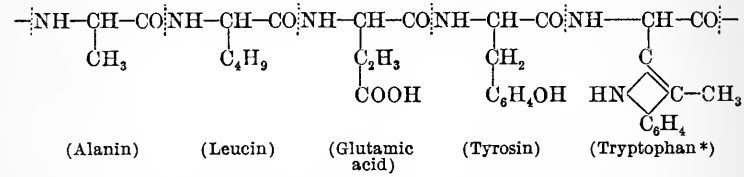
Tetra-peptid :

Tri-glycyl-glycin,
$H_2N-CH_2-CO\!:\!NH-CH_2-CO\!:\!NH-CH_2-CO\!:\!NH-CH_2-COOH$

267. Peptid chains in protein molecules. — The complex peptids resemble in some respects a few of the simplest proteins such as peptones. This synthetic approximation to some natural proteins has led to the belief that the peptid chain is *part* of the structure of the protein molecule and that the protein molecule contains a skeleton comprising parts such as are represented by the following chain-fragment:



The following formula suggests in a general way the manner in which some of the many known cleavage products of proteins may be related to each other in protein molecules:



There is no reason to believe, however, that the protein molecule is a simple chain such as is indicated by the above formula. It is probable that such chains are interlinked in very complex ways and also that, when the molecule is cut up by a process of hydration, the various portions of the interlinked segments are converted into the nuclei of the various amino-acids named on pages 84–86.

The structure that is indicated by the above formulas is at most only the approximate structure of *portions* of protein molecules, but it enables us to understand why it is that, although the true proteins yield so many different cleavage products, they are nevertheless so much the same in their general properties.

268. Every protein is both polybasic and polyacidic. — The protein molecule consists so largely of amino-acid nuclei that proteins themselves show some of the general properties of amino-acids. An amino-acid contains both a carboxyl group and an amino group, and will form salts readily with both acids and bases. Consequently an amino-acid is both acidic and basic in its qualities. But the carboxyl and amino groups counterbalance each other in these respects and amino-acids are practically neutral in reaction.

The relations between a typical amino-acid and forms of the two

* Written as skatol-amino-acetic acid. See page 86.

general kinds of its salts may be summarized in connection with amino-acetic acid in aqueous solution as follows:

Glycocoll: $H_2N—CH_2—COOH$. Practically *neutral*. Dissociation very slight.

Glycocoll hydrochlorid: HCl, $H_2N—CH_2—COOH$. Dissociated into the neutral compound and into $H^{\cdot}$ and Cl' ions. Strongly *acid*.

Sodium glycocollate: $H_2N—CH_2—COONa$. Dissociated into the neutral compound and into $Na^{\cdot}$ and OH' ions. Strongly *alkaline*.

Proteins, by reason of their amino-acid properties are at once *weak acids and weak bases*. Every protein combines with bases and acids to form readily dissociable salts. Some proteins are more acid than basic, or *vice versa*. The glucoproteins represent the former kind, the protamins the latter. All proteins are both polybasic and polyacidic because of the many amino-acid nuclei they contain. These facts account for the variety of combinations into which proteins enter in organisms and in the laboratory (313–321).

G. TYPICAL PROTEINS FOR USE IN THE TESTS.

269. Kinds. In experiments 280–328 use solid samples or the specially prepared solutions of each of the following typical proteins: *albumin* (in white of egg), *edestin* (from hempseed), *acid-albumin* (from meat) and *proteoses* (Witte's — from fibrin). Protein properties that cannot be learned from a study of these products will be noted during the progress of our tissue studies.

270. Preparation. *Crude albumin.* Strike an egg sharply on the edge of a casserole with sufficient force to cut the egg almost in two. Turn the severed egg on end, hinge back the upper portion and remove the white matter to the casserole by transferring the yolk back and forth carefully from one portion of the shell to the other.

271. Temporary disposition of the yolk. Transfer the yolk to the large stoppered bottle. Add to it about 100 c.c. of alcohol. *Stir* the mixture thoroughly and set it aside for future use.

272. Dry egg white. With scissors cut up the membranes in the white matter (270). Transfer about half the material to a porcelain dish and evaporate it to dryness on a water bath at a temperature not above 45° C. *Stir* repeatedly to favor desiccation. After the material has been dried pulverize and bottle it for future use.

273. Crude albumin solution. Egg white contains about 12 per cent. of protein substances. Of these albumin is predominant and the characteristic "albuminous" properties of egg white are due to it.* The associated substances in egg white do not interfere with a study of the properties of the albumin.

274. Prepare a crude albumin solution by treating the remaining portion of egg white as follows: Ascertain the volume of the available supply of egg white (272) and transfer it to a flask. Add to it about 10 volumes of water. Shake the mixture thoroughly and pass the solution through a wet *folded* filter. The filtrate is ready for use.

275. *Edestin.* Mix in a beaker 10 grams of ground hempseed and 100 c.c. of 5 per cent. sodium chlorid solution heated to 60° C. Stir the mixture thoroughly. Place the beaker in hot water in a water bath and maintain, for about 30 minutes, a temperature of 55° to 60° C. The temperature must not be permitted to go above 60° C. After such treatment for 30 minutes filter the mixture on a filtration apparatus wetted with 5 per cent. solution of sodium chlorid. Catch the filtrate in a *large* beaker immersed in hot water (60° C.) in a casserole or water bath. After the filtrate has been collected, heat it on a water bath to 60° C. and add to it sufficient warm water (60° C.) to render the liquid *faintly turbid.* Cover the beaker with a watch glass and set it aside in hot water (60° C.) so that the extract may *cool as slowly as possible.*

276. Edestin solution. After the diluted extract has cooled (275) decant and filter the supernatant liquid, which is *saturated* at room temperature with edestin and may be regarded as a crude edestin solution. As in the case of the albumin solution, the associated constituents do not prevent determination of the general properties of the edestin. Reserve the filtrate for use in the tests.

277. Crystalline edestin. Examine under a microscope the white sediment obtained from the hemp seed extract (276). It consists wholly of edestin, which is usually entirely crystalline — octahedra mainly. Filter the sedimentary mixture. Wash the precipitate with water from the paper into the original beaker. Add a large volume of water to the mixture, and stir thoroughly.

* The generic term that is used by the Germans to designate the primary or true proteins is Eiweisskörper, *i. e.*, egg-white substances (page 77).

After sedimentation, decant the supernatant wash water, filter off the solid matter and wash the latter until it is practically free from chlorid. Use the moist precipitate in the tests.

278. *Acidalbumin.* Place about 10 grams of hashed meat in a large beaker full of water and stir thoroughly to remove blood and soluble matter. Repeat the process several times until the decanted liquid is free from coloring matter. Transfer the washed meat to a small beaker and treat it with about 100 c.c. of 0.2 per cent. hydrochloric acid. Heat as high as possible on a water bath. The acid mixture should be stirred repeatedly. After heating for about 15 minutes filter the mixture and neutralize approximately with dilute potassium hydroxid solution. A precipitate of acidalbumin will be obtained as the reaction of the liquid approaches the neutral point. Filter the mixture, transfer the precipitate to a large amount of water in a beaker, stir thoroughly so as to wash off traces of adherent acid or alkali and, after sedimentation, decant and filter again. Wash the product on the paper until the washings are neutral.

279. *Proteose.* Use "Witte's pepton," a commercial product, consisting chiefly of proteoses with some pepton, obtained from fibrin by hydration methods similar to that shown in demonstration 290.

H. DETECTION OF THE ELEMENTS CONTAINED IN PROTEINS (206).

280. Apply tests 50, 51, 53, 62, 63 and 64 to a sample of one of the protein products.

I. PHYSICAL PROPERTIES OF PROTEINS.

281. Proteins, like carbohydrates, differ considerably both physically and chemically.

282. **Proteins are nonvolatile and do not impart greasy stains to paper (208).**

283. **Most of the pure proteins are neutral compounds (209).**

284. **Microscopic appearance (210).**

285. **Solubilities (211).**

286. **Proteins do not form emulsions (102, 212).**

Demonstrations. 287. Separation of albumin in quantity from egg white and its purification by dialysis. 288. Preparation of crystalline egg albumin. 289. Preparation of gelatinous proteinate. 290. Hydration of primary protein successively to simple secondary protein and to amino-acids, with

methods for the identification of some of the latter. 291. Optical properties of proteins. 292. Ignition of proteins, with an examination of the products of destructive distillation. 293. Putrefaction of proteins, with an examination of the products. 294. Effects on proteins of various kinds of bacteria. 295. Tests of the diffusibility of proteins. 296. Frothiness of protein solutions.

J. COLOR TESTS.

297. Lack of specificity of protein reactions. All proteins respond in common to certain chemical tests. None of the so-called protein tests is strictly characteristic of protein matter, however, for each of the reactions is given by at least one *non-protein* substance. These remarks apply not only to *color* tests but to all others in which proteins may be involved. The very great complexity of the protein molecule and the diversity of its side chains and interlocked nuclei account for the remarkable manner in which proteins share with various other substances certain important reactions.

As a rule any non-protein substance that behaves exactly like protein in a given test fails to simulate protein in any other special reaction. On this account it is possible, through the evidence of a number of corroboratory tests, to determine definitely whether or not protein is contained in a given medium, and also to exclude the influence of non-protein substances. The effects of non-protein substances on protein reactions have been pretty thoroughly studied.

The color reactions that are given by proteins are given by particular nuclei contained in the proteins. A reaction that is given by a particular nucleus in protein matter is given as a rule by any other substance having the same nucleus.

The following special coloration tests (298–307) are given by practically all proteins in solid form or in solution.

Biuret test (L: 252).* **298.** A. To about 5 c.c. of the solution in a porcelain evaporation dish † add sufficient potassium hydroxid to make the solution strongly alkaline. *Do not heat* the mixture for the reason that some proteins are completely decomposed by heat in such alkaline solutions.

* Also occasionally called Piotrowski's test. It was discovered by Rose in 1833.

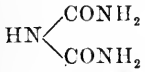
† The porcelain affords a white background and makes it possible to detect the very slight coloration that results when only *traces* of proteins are present. Practically the same result is obtained by making the test in a beaker resting on dry white filter paper.

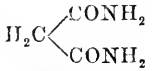
299. B. Prepare a *very dilute* cupric sulfate solution by adding about 2 drops of 2 per cent. solution of cupric sulfate to a common test-tube full of water. The solution should be practically colorless. An excess of cupric sulfate may obscure the characteristic color of the test (227).

300. C. Pour the copper solution (B) gradually into the alkaline solution (A). If protein is present a bluish violet to reddish violet or a bright red or pink coloration results.

301. If the result was positive notice whether a stronger cupric sulfate solution intensifies the color.

The biuret reaction is given by all substances that contain two $-CONH_2$ groups united together or attached to a nitrogen atom or to a carbon atom. Among such substances are the following:

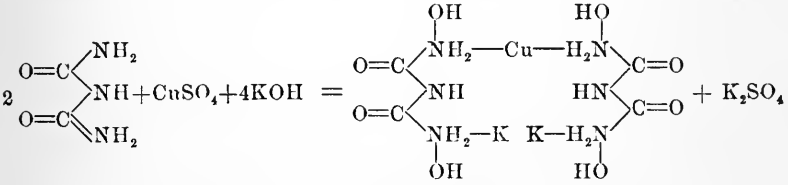

Biuret


Malonamid


Oxamid

That protein will yield biuret or a biuret-like substance on cleavage will seem apparent after comparison of the above formulas with those on page 88 which show the amino-acid nature of parts of the protein molecule.

The color of the test is due to the formation of biuret potassium cupric hydroxid. The equation may be represented as a reaction between biuret and the reagents used, as follows:


Biuret

Biuret potassium cupric hydroxid (soluble: red)

Precautions. Strongly acid solutions should be approximately neutralized before the biuret test is applied to them, otherwise the final alkalinity may not be sufficient to effect transformation of the protein into the colored compound.

The test cannot be applied in the presence of relatively large proportions of substances that react with alkali hydroxid, such as magnesium sulfate and ammonium sulfate. Their removal is necessary before the addition of cupric sulfate (225).

302. Xanthoproteic test.* To about 3 c.c. of the neutral or slightly acid solution in a test-tube add approximately 3 c.c. of concentrated nitric acid. If protein is present the solution will be colored slightly yellowish. Some proteins are precipitated by nitric acid, but their precipitates gradually dissolve, becoming yellowish in the process and yielding yellow matter to the solution. *Boil* for a minute. The yellow color is increased to canary yellow if a moderate amount of protein is present. If a protein precipitate was formed when the acid was added to the solution, the precipitate will be made more yellowish by the higher temperature and will be perceptibly decreased in quantity because of hastened solution.

Cool the acid solution in running water. Pour gently down the side of the tube *after cooling*, † a moderate excess of ammonium hydroxid. If protein was present the color of the alkaline liquid along the line of junction of the two solutions will be deepened to orange. Shake the mixture and thus increase the depth of the layer in which ammonium hydroxid has overcome the acid. The orange band is thereby increased in width and if ammonium hydroxid is present in sufficient excess, the entire solution on complete mixture will be suffused by an orange color.

The reaction is given by many substances, among them aromatic compounds in general. The coloration is due to the formation of various indeterminate aromatic nitro-derivatives. The aromatic nuclei in the protein molecule that are chiefly responsible for the coloration seem to be those of tyrosin and tryptophan (264).

303. Millon's test. Treat about 3 c.c. of the solution in a test tube with approximately 3 c.c. of Millon's reagent.‡ If protein is present a white precipitate may be formed or precipitation may fail to occur, according to the nature of the contained protein. Heat the mixture gently and carry the temperature *slowly* to the boiling

* Among the nitroderivatives of protein matter produced by treatment with concentrated nitric acid is a substance or a mixture of several substances called xanthoprotein. Very little is known about this product. The test was discovered by Fourcroy and Vauquelin in 1805.

† Hot nitric acid and ammonium hydroxid react violently.

‡ Millon's reagent is made as follows: Mercury is dissolved in its own weight of pure nitric acid (sp. gr. 1.4). This concentrated solution is treated with two volumes of water and the diluted mixture is allowed to stand 24 hours. A slight sediment of basic salts will form. The filtrate, containing mercurous and mercuric nitrates, excess of nitric acid and a small amount of nitrous acid, is the reagent.

point. If all of any protein present has been precipitated, the precipitate will assume a red color as the temperature rises and the solution itself will be colorless. The precipitate may dissolve and color the solution red. If unprecipitated protein was present, the solution itself will be reddened.

The reaction is given by all aromatic substances that contain a hydroxyl radical attached to a benzol ring. Thus, it is given by phenol, $C_6H_5 - OH$, but not by benzoic acid, $C_6H_5 - COOH$. The aromatic nucleus in protein matter that seems to be responsible for the coloration is oxy-phenyl-*α*-amino-propionic acid (tyrosin, 264). The nature of the red compound is unknown.

Precautions. Excessive heating should be avoided as a rule. A white precipitate is given by urea, sulfates and other non-protein substances. Alkaline solutions should be acidified slightly with nitric acid before Millon's reagent is added to them, otherwise oxids of mercury may be precipitated from the reagent by the alkali and the reagent rendered valueless. Inorganic salts when present in excess also interfere with the reaction.

304. Hopkins and Cole's modification of Adamkiewicz' test, or the glyoxylic test. To about 2 c.c. of the solution add an equal volume of "reduced oxalic acid solution."* Mix thoroughly. Pour gently down the side of the tube an equal volume of concentrated sulfuric acid. If protein is present a band of purple will form along the line of junction of the two solutions. Shake gently in order to mix the two solutions gradually. If protein is present the entire liquid will be colored purple on mixing the solutions uniformly.

This is a test for tryptophan (264) and is due to the tryptophan nucleus in the protein molecule. The chemical character of the colored product is unknown.

Precautions. The reaction is prevented by an excess of chlorids and by certain salts that are not conspicuous in biological liquids, such as nitrates. Various carbohydrates, such as sucrose, are converted into black or colored products by concentrated sulfuric acid

* "'Reduced oxalic acid' is prepared by treating half a liter of a saturated solution of oxalic acid with 40 grams of 2 per cent. sodium amalgam in a tall cylinder. When all the hydrogen has been evolved the solution is filtered and diluted with twice its volume of water. The solution now contains oxalic acid, sodium binoxalate, and glyoxylic acid (COOH—CHO). It should be kept in a closed bottle containing a little chloroform." [Cole.]

and they accordingly interfere with the test by obscuring the characteristic color. Pure concentrated sulfuric acid is essential — some common impurities in ordinary commercial sulfuric acid may prevent the reaction.

305. **Liebermann's test** (310). Treat a small amount of solid protein matter with a moderate quantity of alcohol and heat the mixture for about 15 minutes on a water bath to effect thorough dehydration of the protein. Transfer the protein matter, after filtration, to a small amount of ether in a test-tube for removal of adherent fat. Shake repeatedly. After exposure to the ether for about an hour or more, transfer the dry protein to a small beaker, add to it a moderate quantity of concentrated hydrochloric acid and boil vigorously for several minutes. The protein dissolves. A deep blue or violet blue is the characteristic color of the test. The nature of the colored substance is unknown.

The reaction is regarded as a furol test and is thought to be due to the simultaneous presence in the molecule of a carbohydrate nucleus and an oxy-phenyl group. It may also be due to tryptophan in reaction with glyoxylic acid (304) from the ether employed.

306. **Molisch's test.** Apply the test as suggested in section 222. A positive result indicates the presence of a carbohydrate nucleus.

307. **Test for loosely combined sulfur** (58, 59, 55). A positive result depends upon the production of hydrogen sulfid from mercaptan groups in the molecule. The mercaptan groups are contained in the nuclei of sulfurous cleavage products, such as cystin and others referred to on page 85.

Demonstrations. 308. Conditions that interfere with the color reactions of proteins. 309. Effects of iodin on proteins (115, 218). 310. Effects of cold and hot concentrated mineral and organic acids and alkalies on proteins (315). 311. Production of carbohydrate material from a protein (tendomucoid). 312. Precipitation of proteins by agitation.

K. PRECIPITATION OF PROTEINS.

313. We have already learned that in the free state practically all proteins are neutral compounds. We have also noted the fact that, like amino-acids, all proteins are at once polyacid and polybasic in the presence of acids and bases (268). In the free state most of the proteins dissolve in water without undergoing hydro-

lytic dissociation. Water is the most general protein *solvent*. In the presence of acids and bases, *i. e.*, ions, the dissolved protein is *ionized* and it forms salts that may be soluble or insoluble according to the nature and effects of the non-protein ions. Proteins may, therefore, be anions or cations according to the electrical conditions of the ions that are introduced into solution with them.

Determine the precipitative effects upon protein solutions of the reagents indicated below.

314. Alcohol. (240). Alcohol is one of the most general of protein precipitants. All proteins are insoluble in absolute alcohol. Some proteins are soluble in 95 per cent. alcohol, however, and a greater number are soluble in more dilute alcohol. Consequently some proteins are difficult to precipitate from aqueous solution with alcohol. The minimal degree of concentration for precipitation differs greatly for the various groups of proteins.

The precipitate that is thrown from aqueous protein solutions by alcohol is the protein itself as it existed in solution. The alcohol does not combine with it. Some proteins are quickly rendered insoluble by alcohol, after their precipitation, probably by a dehydration process. Other proteins do not seem to be affected chemically in any way by alcohol under such conditions.

315. Strong mineral acids — *Nitric, hydrochloric, sulfuric.* Pour protein solutions gently down the sides of the tubes upon the acids in order to make "ring tests." Notice the white precipitates that may be produced. Shake the mixtures gently to effect complete mixture gradually.

316. Notice the effects of excessive additions of the acids (315). Boil half of each solution. Set aside the boiled and the cold portions for later observation of the colorific effects (310). The production of various colors from the same colorless material by different agents suggests the same possibility in organisms. Many of the coloring matters in organisms are biological cleavage products of proteins.

317. Saturation with neutral salts — *Sodium chlorid, magnesium sulfate, ammonium sulfate* (243). Add the finely powdered salts to small quantities of protein solutions in *beakers*. Avoid unnecessary excesses of the salts. After each solution has been saturated, pour it into a narrow test-tube and notice whether a protein precipitate has been produced.

318. Acidify with acetic acid any of the solutions that may *not* have been precipitated by saturation with a salt (317). Notice that acetic acid alone will not precipitate to the same degree, if at all, any of the *original* protein solutions.

319. Filter any of the solutions that may have been precipitated by saturation with a salt (317) and apply to the filtrate in each case the biuret reaction (301).

Ammonium sulfate, when added to full saturation of their aqueous solutions, completely precipitates all forms of protein matter except peptones and some proteoses (243).

The protein precipitates that are produced by saturation with neutral salts are protein-saline compounds.

The effects just noted are comparable to the process of "salting out" soaps (129) and polysaccharids (245).

320. **Salts of heavy metals** — *Silver nitrate, mercuric chlorid, plumbic acetate, cupric sulfate, ferric chlorid.* Notice the effects of moderate proportions and of excesses of the reagents.

Each precipitate consists of a compound of a metallic cation and a *protein anion.* Cations of the heavy metals completely precipitate most of the proteins from neutral, acid and alkaline solutions.

321. **Alkaloidal reagents** — *Phosphotungstic acid, tannic acid, picric acid, trichloracetic acid, potassio-mercuric iodid, potassium chromate, hydroferrocyanic acid.* Apply the reagents after acidification of the protein solution with dilute hydrochloric acid. In the last test (hydroferrocyanic acid), acidify the solution with acetic acid and add potassium ferrocyanid. Hydroferrocyanic acid results * and immediately acts upon the protein.

Each precipitate consists of a compound of a reagent anion and a *protein cation.* Anions of the alkaloidal reagents completely precipitate most of the proteins in *acid* solutions. In neutral or alkaline solutions protein compounds with the alkaloidal reagents are, as a rule, completely dissociated and remain in solution. Bases that are as weak as most of the proteins do not form precipitates with these reagents in the absence of an excess of $H^{\cdot}$ cations.

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* Equation: $K_4Fe(CN)_6 + 4CH_3COOH = H_4Fe(CN)_6 + 4CH_3COOK$
Potassium ferrocyanid — Hydroferrocyanic acid

L. COAGULATION OF PROTEINS.

322. Albumins, globulins, proteinates, and a few of the secondary (compound) proteins that contain albumin- or globulin-like nuclei, may be rendered completely insoluble, *i. e.*, *coagulated*, if they are heated while dissolved or suspended in neutral or faintly acid liquids. The high temperature causes a permanent chemical alteration which is not yet understood. In the coagulation process a *very slight* proportion of alkaline reacting material (NH_3?) is thrown out of the molecule, intramolecular rearrangement occurs, and in neutral or faintly acid liquids all of the coagulable protein separates as a flocculent precipitate (coagulum). The weight of the coagulum, in carefully conducted experiments, is practically the same as that of the original protein (330). There is no way by which the coagulum can be reconverted into the original protein. Coagulation is a *physical* expression of a *permanent* though relatively slight *chemical* alteration. Coagulable proteins have *specific* coagulation temperatures.

323. **Coagulation of solid primary proteins.** Suspend in water or 10 per cent. salt solution albumin, globulin, or acidalbumin. Boil the mixture for a minute or two. Filter. Observe that the solid substance no longer dissolves in some of the reagents that had previously been found to dissolve it readily, *e. g.*, 0.2 per cent. hydrochloric acid (285).

324. **Coagulation of dissolved primary proteins.** To about 10 c.c. of clear neutral solutions of albumin (H_2O) and edestin (5 per cent. NaCl) in small beakers add 20 c.c. of water. Boil. Observe the opalescence that is produced as the boiling points are neared. Test the reactions of the hot liquids.* Add to each several drops of very dilute acetic acid.† Notice the immediate flocculation that results. Again test the reaction of each liquid. Add excess of dilute acid, *e. g.*, 0.2 per cent. hydrochloric acid (328).

Non-coagulability of proteinates in acid or alkaline solvents

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* In the coagulation process a very slight amount of alkaline reacting material is produced from the proteid (322). It may be too slight for detection with an ordinary indicator, without special treatment of the liquid.

† Prepare very dilute acetic acid by allowing a single drop of common 36 per cent. acetic acid to fall into an ordinary test-tube full of water. Shake thoroughly. Use this very dilute solution in the test referred to above.

and also of proteoses (representing groups A, b, 1 and 3 of the simple secondary proteins). 325. Repeat the last experiment (324) with acid (0.2 per cent. HCl) or alkaline (0.5 per cent. Na_2CO_3) solutions of acidalbumin.

326. Repeat with proteose experiments 323 and 324.

Determinations of temperatures of coagulation and of the effects on coagulability (and its temperatures) of the (*A*) concentration of the protein, of the (*B*) reaction of the solvent and of (*C*) associated saline matter.

327. Prepare in *narrow* test-tubes of *equal size* the following solutions: *

A. Influence of concentration of the protein.

1. 5 c.c. of *neutral* dilute albumin solution (1 part of egg white + 39 parts of water).
2. 5 cc. of *neutral* concentrated albumin solution (1 part of egg white + 9 parts of water).
3. Solution 2 + 5 c.c. of water (1 part of egg white in 20).

B. Influence of the reaction of the solvent.

4. Solution 2 + 5 *drops* of 0.2 per cent. hydrochloric acid.
5. Solution 2 + 5 *drops* of 0.5 per cent. sodium carbonate.
6. Solution 2 + 1 *drop* of 36 per cent. acetic acid.
7. Solution 2 + 1 *drop* of 10 per cent. potassium hydroxid.

C. Influence of associated saline matter.

8. Solution 2 + 5 c.c. of 5 per cent. sodium chlorid.
9. Solution 2 + 5 c.c. of 10 per cent. sodium chlorid.

328. Subject the solutions (1–9) to the following treatment: Support any one of the tubes with a clamp attached to the iron rod. Place a thermometer in the clamped tube and fasten, with a rubber band, all the other tubes to the one in the clamp. Immerse the tubes in cold water in a large beaker on a tripod. The ends of the tubes should not touch the bottom of the beaker and the level of the liquid in each tube should be below the level of the surrounding water. Do not transfer the thermometer from one tube to another during the course of the experiment. It may be safely presumed that the tem-

* The neutral albumin solutions were prepared in bulk as follows: Solutions of egg white were made in the proportions indicated above: "Dilute," 1 in 40; "concentrated," 1 in 10. The reaction of fresh egg white is slightly though quite distinctly *alkaline* (phosphates). Each of the two solutions was carefully neutralized with very dilute acetic acid, warmed to the temperature of the body (37° C.) and filtered.

perature will rise uniformly in all the tubes. Heat the water in the beaker with a low flame. Stir the water frequently. Carry the temperature *gradually* to the boiling point of the water and notice the temperature at which *initial coagulation* occurs, *i. e.*, the temperature at which the faintest possible turbidity can be detected. Be especially alert after the temperature has reached 50° C. Coagulation will begin almost simultaneously in several of the tubes. Do not detach any of the tubes until after the surrounding water has reached the boiling point.

Salts and *traces* of free acid in the solvents favor coagulation of proteins that are coagulable by heat. They lower the temperature of coagulation. Heat coagulation is impossible in the absence of salts. It is prevented by alkali, even in very minute proportions, and also by acid when the latter is present in appreciable quantity. The preventive influence of alkalinity is greater than that of acidity. No proportion of alkalinity is favorable to the process.

Acid or alkali converts albumins and globulins into proteinates like acidalbumin. The *proteinates are noncoagulable when they are dissolved in acid or alkaline liquids*, but they may be coagulated, as we have seen (323), if heated while they are *suspended* in *neutral* fluids.

Coagulated protein is practically insoluble in water, saline solution, dilute acid and dilute alkali (329).

Acid or alkali, when present in cold protein solutions, tends to convert coagulable proteins into proteinates. Heat greatly facilitates the process. When such acid or alkaline solutions are heated coagulable proteins may be entirely converted into proteinate *before* the solution reaches the temperature of coagulation of the original protein. When the proportion of acid or alkali is insufficient to convert all of the coagulable protein into proteinate by the time the coagulation temperature of the former is reached, the untransformed portion of the original protein will, at that point or at a somewhat higher temperature, be thrown from solution as flocculent coagulum.

If coagulable protein is dissolved in a *neutral* or *very faintly acid* solution (containing saline matter) the addition of a slight amount of acid, *after* the boiling point has been reached, transforms the turbid opalescent solution to a flocculent mixture (324), the precipitated

protein is coagulated in the process and an excess of dilute acid or even of dilute alkali is then unable to dissolve it.

Demonstrations. 329. Properties of coagulated protein. 330. Method for the quantitative determination of coagulable protein. 331. Methods for the detection of protein in the presence of fatty products and carbohydrates. 332. Methods for the separation of fats and fatty products, carbohydrates and proteins when present together in a mixture.

333. Percentage amounts of proteins obtained from some mammalian parts and from various vegetable food-stuffs (236).*

The figures in the subjoined table represent general average percentage amounts of proteins contained in the materials named:

Animal parts.		Blood:	
Urine	Trace	Corpuscles	31.0
Saliva	0.3	Plasma	8.0
Gastric juice	0.3	Tendon	34.7
Adipose tissue	0.9	Ligament	40.0
Bile	2.5		
Milk	4.0	Vegetable foods.	
Lymph	4.1	Cucumber	1.0
Liver	5.8	Potato	2.0
Brain	7.3	Spinach	3.1
Pancreatic juice	8.0	Apple	4.0
Bone (shaft)	20.0	Rice (grains)	7.0
Cartilage	25.0	Wheat (grains)	12.3
Muscle	26.5	Peas	22.0

*The proportions of proteins in some of the products named in the table vary considerably under ordinary conditions.